VOLUME 67 *March, 1953* NUMBER 3

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

CONTENTS

Trend of Tuberculosis Morbidity. JULIUS KATZ 279
The Response to Antimicrobial Therapy of Anergic and Partially Anergic Tuberculous Patients: I. Changes in the Tuberculin Sensitivity of Individual Patients. C. EUGENE WOODRUFF, WILBUR J. STEININGER, RUBY G. KELLY, AND HERBERT KREISEL 286
The Response to Antimicrobial Therapy of Anergic and Partially Anergic Tuberculous Patients: II. The Prolongation of Life. HERBERT KREISEL, WILBUR J. STEININGER, W. LEONARD HOWARD, EDWIN E. MUELLER, AND C. EUGENE WOODRUFF 292
Electrophoretic and Chemical Serum Protein Fractions in Pulmonary Tuberculosis. BRUNO W. VOLK, ABRAHAM SAIFER, LINDEN E. JOHNSON, AND IRWIN ORESKES 299
The Bactericidal Action of Isoniazid, Streptomycin, and Terramycin on Extracellular and Intracellular Tubercle Bacilli. G. B. MACKANESS AND N. SMITH 322
Irradiated Antituberculosis Vaccine, Including Comparison with BCG in Experimentally Infected Guinea Pigs. JOSEPH B. SEAGLE, ALFRED G. KARLSON, AND WILLIAM H. FELDMAN 341
Chemotherapy of Experimental Tuberculosis. VI. Derivatives of Isoniazid. JACK BERNSTEIN, WILLIAM P. JAMBOR, W. A. LOTT, FELIX PANSY, BERNARD A. STEINBERG, AND HARRY L. YALE 354
Chemotherapy of Experimental Tuberculosis. VII. Heterocyclic Acid Hydrazides and Derivatives. JACK BERNSTEIN, WILLIAM P. JAMBOR, W. A. LOTT, FELIX PANSY, BERNARD A. STEINBERG, AND HARRY L. YALE 366
Case Reports:
Histoplasmosis: Report of Two Cases. ROBERT CHARR 376
Peliosis Hepatis. Report of Two Cases. J. M. YOUNG 385
Notes:
Lack of Significant *in Vitro* Sensitivity of *Mycobacterium Tuberculosis* to Pyrazinamide on Three Different Solid Media. MAURICE S. TARSHIS AND WILLIAM A. WEED, JR. 391
American Trudeau Society:
Notes 396
Obituaries 398
Abstracts 49

ANNUAL MEETING—LOS ANGELES, CALIF.
May 18–22, 1953

See inside back cover

Published Monthly **at Mount Royal and Guilford Avenues, Baltimore 2, Maryland by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 10, N. Y.**

Copyright 1953, by the National Tuberculosis Association

PRINTED IN U. S. A.

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY

Medical Section of National Tuberculosis Association

EDITOR

WALSH McDERMOTT

New York, N. Y.

CONSULTING EDITOR

ESMOND R. LONG

Philadelphia, Pa.

EDITOR OF ABSTRACTS

LAWRENCE B. HOBSON

New York, N. Y.

Helen W. Miller, *Editorial Secretary*

EDITORIAL BOARD

John D. Adcock, M.D., Ann Arbor, Mich.
William H. Feldman, D.V.M., Rochester, Minn.
Byron F. Francis, M.D., Seattle, Wash.
Max B. Lurie, M.D., Philadelphia, Pa.
Donald L. Paulson, M.D., Dallas, Tex.
Philip E. Sartwell, M.D., Baltimore, Md.
John D. Steele, M.D., Milwaukee, Wis.
William B. Tucker, M.D., Minneapolis, Minn.
Arthur J. Vorwald, M.D., Saranac Lake, N. Y.

BUSINESS MANAGER

HENRY W. STEVENS

NOTICE TO SUBSCRIBERS AND CONTRIBUTORS

THE AMERICAN REVIEW OF TUBERCULOSIS is published by the National Tuberculosis Association and issued monthly about the first of the month. THE AMERICAN REVIEW OF TUBERCULOSIS is the official journal of the American Trudeau Society. A volume includes six numbers and begins with the January and July numbers.

Subscription: The subscription price of the REVIEW is $12 for the calendar year. Checks should be made payable to Collier Platt, Treasurer. The price of single copies is $1.50 postpaid.

Character of the Review: The REVIEW invites the submission of original manuscripts on any phase of tuberculosis, nontuberculous pulmonary diseases, and related subjects of interest.

MANUSCRIPTS

Contributors are requested to pay particular attention to the following rules governing the preparation of manuscripts. Failure to follow these rules is a frequent cause of delay in the publication of articles.

Manuscripts should be sent in duplicate to the office of the Editor, Dr. Walsh McDermott, 1790 Broadway, New York 19, N. Y., mailed flat, and transmitted by first-class mail with postage for return if not acceptable. They should be in English, double-spaced, typewritten on one side of the page with wide margins. Tabular data should not be included in the text but should be presented in separate tables with appropriate reference in the text. All cases of pulmonary tuberculosis should be classified according to the 1950 edition of *Diagnostic Standards and Classification of Tuberculosis* published by the National Tuberculosis Association. All case reports should be written in complete sentences. Authors should exercise particular care in the preparation, notation, and description of figures and tables. The form of reference used in the REVIEW should be carefully followed and references should be checked for accuracy. Each table must be accompanied by a descriptive title and a suitable legend must be provided for each figure. Drawings must be made with india ink and all lettering should be made with a stencil. The quality of the reproduction of a roentgenogram depends upon both the quality of the original picture and of the glossy print obtained from photographing that picture. If a contributor does not have facilities available, preparation of figures or reproduction of roentgenograms may be handled through the REVIEW office. In those circumstances, the contributor should supply a neat, properly proportioned, and suitably entitled drawing or the original roentgenogram, and will be expected to defray the cost of these services.

The publishers will not be responsible for manuscripts, illustrations, etc., lost in transit. In order to save expense for authors' corrections in proof, manuscripts should be carefully revised by the author before submission.

Reprints: Fifty reprints without covers of articles will be furnished to authors free of charge when requested in advance. Additional reprints may be ordered.

Advertising: Rates will be furnished by THE AMERICAN REVIEW OF TUBERCULOSIS, 1790 Broadway, New York 19, N. Y. on request.

The Publishers reserve the right to decline any advertising submitted and to censor all copy. Acceptance of an advertisement does not constitute official endorsement of the product advertised.

Entered as second-class matter March 9, 1917, at the post office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at a special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 2, 1918.

When Fever Persists . . .

. . . physicians often prescribe one or more Pulvules 'Becotin with Vitamin C' a day. This potent formula provides, in one pulvule, a *therapeutic dose of all the better-known water-soluble vitamins.*

Each pulvule contains:

Thiamin Chloride	10	mg.
Riboflavin	10	mg.
Pyridoxine Hydrochloride	5	mg.
Nicotinamide	50	mg.
Pantothenic Acid (as Calcium Pantothenate)	25	mg.
Vitamin B_{12} (Activity Equivalent)	1	mcg
Ascorbic Acid	150	mg.
Liver Preparation and Stomach-Tissue Material, Desiccated, Lilly	0.39	Gm.

Eli Lilly and Company
Indianapolis 6, Indiana, U. S. A.

Lilly

For metabolic support in febrile diseases prescribe

PULVULES

'Becotin with Vitamin C'

(VITAMIN B COMPLEX WITH VITAMIN C, LILLY)

Meat...

and the Weight Reduction Diet in Cardiac Disease

The important relationship between obesity and the outlook in cardiac disease and hypertension is vividly emphasized in a recent publication of The American Heart Association.*

For reasons not entirely understood at present, "heart disease and high blood pressure are more common in overweight persons than in those of desirable weight." The predisposition to atherosclerosis in obesity and the increased physical burden of carrying excess weight are undoubtedly contributing factors. Hence, as this publication points out, weight reduction is the first line of defense in decreasing the incidence of cardiac disease, and in improving the prognosis after cardiac disease or hypertension has developed.

Meat occupies a prominent position in the weight reduction diets outlined in this American Heart Association booklet. This recommendation is in sharp contrast to the erroneous belief held in former years that meat is harmful in hypertension or cardiac disease. "There is no evidence that red meat or any other form of protein in moderation has any adverse influence on blood pressure."

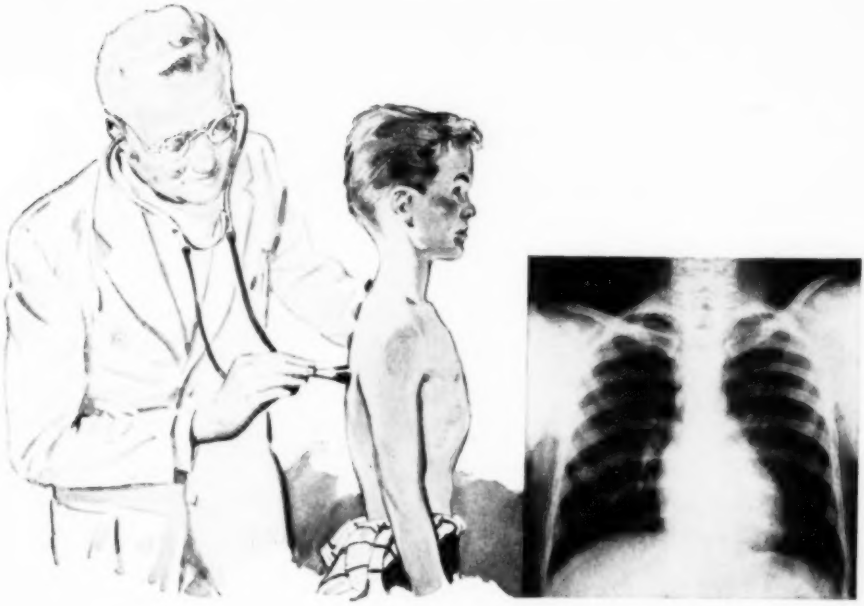
The magic formula for reducing is simply "Eat less." Two types of diets are outlined. One "allows moderate amounts of meat and other proteins, small amounts of fat and moderate amounts of carbohydrates." The other is "high in protein with plenty of meat, eggs and cheese, moderate in fat and low in carbohydrates." Diet No. 1 provides 70 Gm. of protein, 60 Gm. of fat, and 120 Gm. of carbohydrate; caloric yield, 1,300. Diet No. 2 provides 100 Gm. of protein, 80 Gm. of fat, and 60 Gm. of carbohydrate; caloric yield, 1,360.

The inclusion of generous amounts of meat in these diets—12 to 16 ounces of cooked meat or two substantial servings each day in Diet No. 2—is a reflection of the important role meat plays in any weight reduction regimen. It is generously included because of its high content of protein of excellent biologic value and because lean meat contains unobjectionably small amounts of fat.

*Food For Your Heart, a Manual for Patient and Physician, Department of Nutrition, Harvard School of Public Health, Harvard University, The American Heart Association, Inc., New York, 1952. Copies available through local Heart Association.

The Seal of Acceptance denotes that the nutritional statements made in this advertisement are acceptable to the Council on Foods and Nutrition of the American Medical Association.

American Meat Institute
Main Office, Chicago . . . Members Throughout the United States



A drug of choice in tuberculosis

As therapeutically active as streptomycin, CRYSTALLINE DIHYDROSTREPTOMYCIN SULFATE MERCK is less toxic to the vestibular apparatus, minimizes pain and swelling on injection, and may be used even in some patients allergic to streptomycin.

This preferred product is available in dry powder form and in convenient ready-to-inject form as SOLUTION OF CRYSTALLINE DIHYDROSTREPTOMYCIN SULFATE MERCK.

PARA-AMINOSALICYLIC ACID MERCK (PAS), when used in combination with CRYSTALLINE DIHYDROSTREPTOMYCIN SULFATE MERCK, prolongs the effective period of antibiotic therapy by inhibiting or delaying the development of bacterial resistance.

Crystalline Dihydrostreptomycin Sulfate Merck

COUNCIL ACCEPTED

Research and Production for the Nation's Health

MERCK & CO., INC.
Manufacturing Chemists
RAHWAY, NEW JERSEY

by Pilling

PRECISION INSTRUMENTS FOR

THORACIC SURGERY

Improved models... New designs

Th 857 Deep Thoracic Scissors—12" Long, Angular

Long enough to keep the operator's hands out of the field of vision yet as easily handled as small scissors. Degree of angulation is useful in any situation but not so great as to be awkward in a small space. Safer than curved scissors for cutting vascular tissue.

Th 227A Rienhoff Modified Finochietto Rib Spreader

Lighter and less bulky than standard models. Particularly useful in children, young adults and thin individuals where the regular Finochietto retractor may be somewhat cumbersome. Also useful as a sternum spreader.

4 blade sizes, all with 6" spread between blades.

Th 992 Finochietto Thoracic Forceps

Double Utility Clamp—Mixter Type with Longitudinal Serrations and Easily-Threaded, Smoothly-Ground Eye.

Has three useful functions: as a ligature carrier, a hemostat or a dissector. Of particular value in ligating vessels encountered during surgery of the esophagus. Length 25 cm.

Th 1118 Finochietto Needle Holder

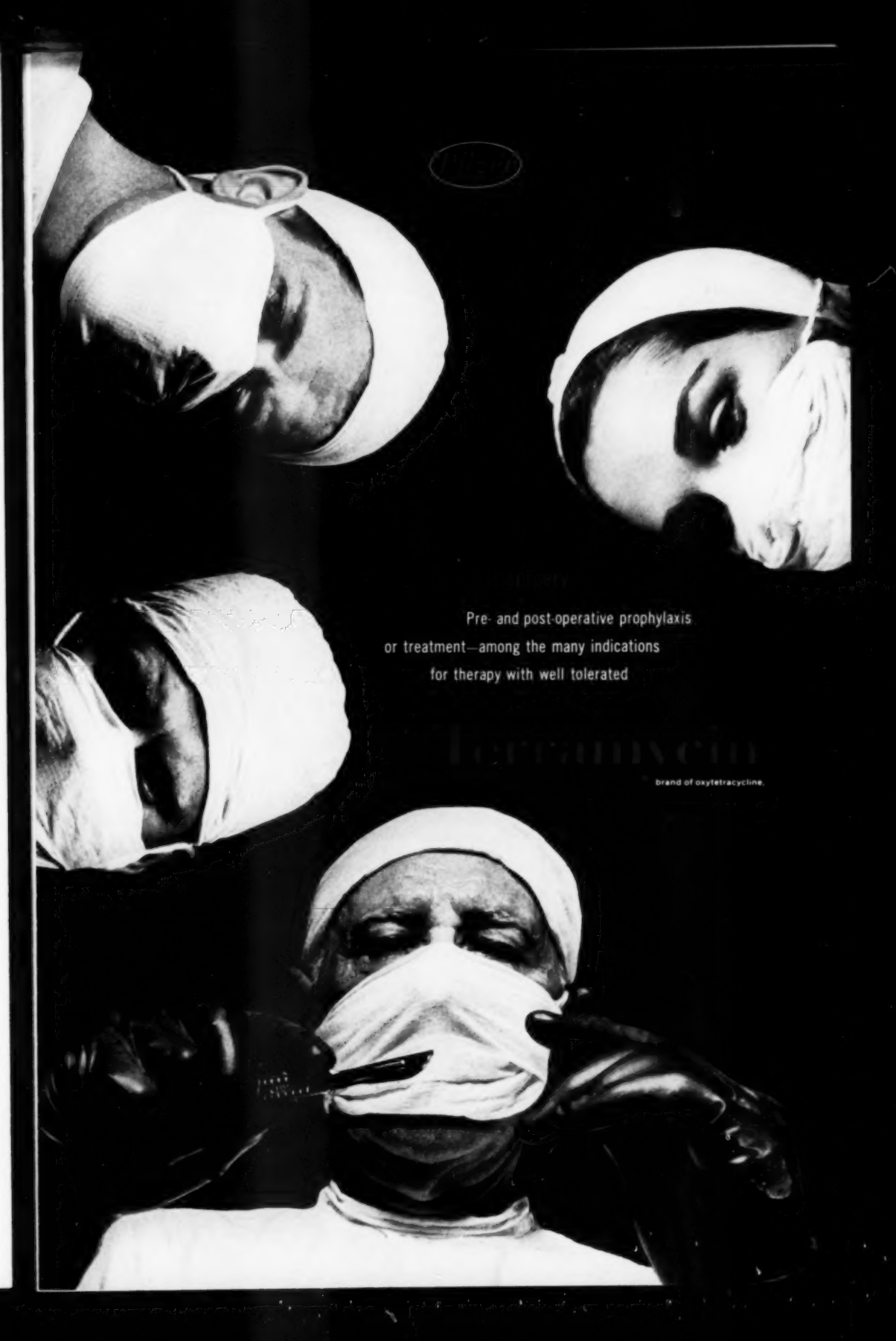
Angulation of tip facilitates suturing in deep areas in chest and abdomen. Can be turned at various angles to permit accurate placing of sutures when performing anastomoses. Particularly useful for esophago-gastric anastomoses deep in the chest at difficult angles.

Order direct from

GEORGE P. Pilling & SON COMPANY

3451 WALNUT STREET • PHILADELPHIA

HEADQUARTERS FOR INSTRUMENTS FOR THORACIC SURGERY


Pre- and post-operative prophylaxis
or treatment—among the many indications
for therapy with well tolerated
brand of oxytetracycline.

THE Panray CORP.

Notes on TUBERCULOSIS CHEMOTHERAPY

March, 1953

PAS THERAPY WITH MINIMUM SIDE EFFECTS

Many hospitals and TB specialists have obtained gratifying clinical response with BUFFERED PARASAL® tablets—an exclusive formulation of para aminosalicylic acid that (1) minimizes GI upsets and PAS intolerance, (2) contains no sodium or sugar, (3) permits *2 tablets to replace 3* of the usual sodium PAS, and (4) is consequently more convenient and economical to administer.

The unique buffering action is non-systemic, effectively maintains stomach pH at optimal pepsin activity for 2 to 3 hours, and avoids the "acid rebound" that often occurs after ingestion of systemic antacids.

Product No. 744.

PARASAL-INH . . . COMBINATION THERAPY IN 1 TABLET

Isoniazid is now finding its place in combined therapy with PARASAL and streptomycin. For more convenient administration, we have developed a combination tablet, BUFFERED PARASAL-INH; each tablet contains 0.5 Gm. PARASAL and 12.5 mg. ISONIAZID 'PANRAY.' (For effect of buffering action, see "A" above.)

Here are the dosage equivalents of the new tablet:

Buffered PARASAL-INH total daily tablets:	Yields total daily PAS	ISONIAZID
16	8 Gm.	+ 200 mg.
18	9	+ 225
20	10	+ 250
24	12	+ 300

Both PARASAL and ISONIAZID 'PANRAY' are AMA Council Accepted, but the *combination* is still on trial and is available for investigational use only.

Product No. 790.

STREP-INH COMBINATION THERAPY

STREPTONIAZID, a sterile solution of dihydrostreptomycin and ISONIAZID 'PANRAY' is another Panray drug developed to make combination therapy as simple and as foolproof as possible. This parenteral form is packaged in 4-cc vials, each containing 1 Gm. of dihydrostreptomycin and 150 mg. of ISONIAZID.

Because the combination is quite new, STREPTONIAZID like BUFFERED PARASAL-INH described above, is available for investigational use only.

Product No. 780.

Additional information is available for all three Panray products discussed above.

Research and Production for the Medical Profession

THE Panray CORP.
340 Canal Street, New York 13, N. Y.

If you make one chest film a day . . . or do hundreds —

LOOK TO GE

14 x 17 inch film cassette channel

Camera back for 4 by 5 inch cut film

Camera back for 4 by 10 inch cut film

Camera for 70 mm roll film

Select your photoroentgen unit from the complete General Electric line

Whatever your TB case-finding program — there's a GE photoroentgen unit to fill your exact diagnostic requirements.

The Single-Stand Model shown above can be used in conjunction with your existing facilities. Or you can get the Duplex — a complete, self-contained model, furnished as an institutional or mobile unit.

With any model, you can choose from four camera backs to get the size and type of film best-suited to your program. And with all GE photoroentgen units, you are assured of time-proved GE results: high-fidelity, long-life performance.

See your GE x-ray representative for complete information, or write X-Ray Department, General Electric Company, Milwaukee 1, Wisconsin, for Pub. S-3.

You can put your confidence in —

GENERAL ELECTRIC

Announcing

a new antibacterial combination

Gantricillin is the new combination of Gantrisin 'Roche' (the single, more soluble sulfonamide) plus penicillin.

Gantricillin is recommended for infections susceptible to penicillin or sulfonamides. It is especially useful when the causative organisms are more susceptible to the combination than to either drug alone. Each scored tablet contains 0.5 Gm Gantrisin and 100,000 units of crystalline penicillin G potassium.

Hoffmann-La Roche Inc., Nutley 10, N. J.

GANTRISIN®—brand of sulfisoxazole
GANTRICILLIN™

THE AMERICAN REVIEW
OF
TUBERCULOSIS

VOLUME 67 *March, 1953* NUMBER 3

TREND OF TUBERCULOSIS MORBIDITY[1]

JULIUS KATZ

(Received for publication September 8, 1952)

INTRODUCTION

In contrast to well-documented evidence of the marked decrease in mortality from tuberculosis during the past fifty years or more, there is a dearth of reliable information regarding morbidity from this disease. The number of deaths and reported cases and the ratio between the two are used, therefore, to estimate the trend of morbidity. Recent reports based upon this type of statistical data conclude pessimistically that, while the number of deaths and the death rates from tuberculosis have decreased considerably since 1900, the rate of development of new cases of this disease has not changed or is increasing.

Edwards and Drolet (1) state that "a widening gap is developing between the tuberculosis morbidity and mortality in the United States"; while Drolet and Lowell (2) conclude that "it is primarily institutional care, or rather improved medical and surgical treatment of active cases of tuberculosis, which is now modifying so favorably the mortality record. Where tuberculosis campaigns remain weak, however, is still in the field of prevention with regard to reducing outbreaks of new cases of tuberculosis."

The implications of these statements are so important as to compel further study and closer analysis of available morbidity data since the logical conclusions to be drawn from them are that tuberculosis is mainly a clinical problem and that tuberculosis control programs have failed in their most important function, the prevention of spread of the disease. It is the purpose of this paper to review some of the available morbidity data in an effort to determine the trend of the incidence of tuberculosis for Upstate New York (New York State exclusive of New York City).

MORTALITY

Before considering morbidity, however, the effect upon the reduction in death rate of institutional care, or of improved medical and surgical treatment of active cases of tuberculosis, needs some consideration. In order to make it clear that

[1] From the Division of Tuberculosis Control, New York State Department of Health, Albany, New York.

other factors besides those of hospitalization and treatment are involved in the declining death rate, it is only necessary to point out that the decline is not a phenomenon of recent origin. In Upstate New York, for example, facilities for the hospitalization of the tuberculous were quite limited until approximately 1915, when a number of county sanatoriums were constructed. Moreover, during the early years of this century the treatment of tuberculous patients was nonspecific in character, being limited mainly to bed rest, with pneumothorax and surgery being used infrequently. Nevertheless, in spite of inadequate facili-

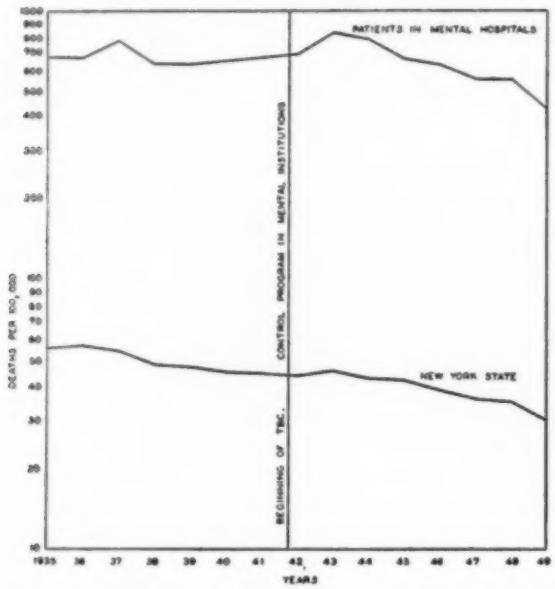

PATIENTS IN MENTAL HOSPITALS
CONTROL PROGRAM IN MENTAL INSTITUTIONS
BEGINNING OF TBC.
NEW YORK STATE
DEATHS PER 100,000
YEARS
1935 36 37 38 39 40 41 42 43 44 45 46 47 48 49

FIG. 1. Deaths from tuberculosis (all forms) for patients in mental hospitals and for New York State.

ties for segregation and the limitations in treatment, there was a continuous decrease in the death rate. Furthermore, even after specific therapeutic measures such as pneumothorax and surgery came into common usage, the decrease in the death rate has been fairly regular since 1900, without periods of rapid sharp decline. This suggests that until quite recently the percentage of patients who received such specific treatment for tuberculosis was too small to produce a significant effect upon the death rate. The use of antimicrobial drugs and excisional lung surgery may have a considerable effect upon the rapidity of decline of the death rate, but more time will be required before this can be determined.

For additional evidence that the decrease in the tuberculosis death rate is not entirely dependent upon the results of specific therapy of tuberculous patients, the experience of the past ten years in the control of tuberculosis among patients

in the institutions of the Department of Mental Hygiene may be cited. During the ten-year period beginning with 1941, the rate of decrease of tuberculosis mortality among these patients has been quite similar to that for New York State as a whole (table 1, figure 1), even though the use of antimicrobial drugs and collapse therapy was very limited during this period. Since the rate of decline of mortality from tuberculosis is lower among those in older age groups than among younger persons, this similarity in change in death rate among mental patients (whose average age is approximately 52) to that in the general population (with an average age of approximately 35) is an indication that

TABLE 1

DEATHS FROM TUBERCULOSIS (ALL FORMS)

Rates per 100,000 for Patients in Mental Hospitals, and for New York State

YEAR	MENTAL HOSPITALS		NEW YORK STATE	
	Number of Deaths	Rate per 100,000	Number of Deaths	Rate per 100,000
1935	409*	676.9*	7,265	55.6
1936	418*	667.2*	7,477	56.9
1937	501*	769.7*	7,171	54.2
1938	427*	639.5*	6,403	48.1
1939	433*	633.3*	6,353	47.4
1940	461	655.5	6,095	45.1
1941	482	671.0	6,111	45.0
1942	508	688.8	5,976	43.7
1943	608	839.7	6,281	45.5
1944	561	783.8	5,997	43.0
1945	477	662.3	5,907	42.1
1946	467	635.9	5,525	38.9
1947	402	558.3	5,214	36.2
1948	410	553.1	5,078	34.7
1949	342	433.8	4,389	29.8

* Estimated.

factors other than specific treatment are also involved in the reduction of deaths from tuberculosis.

It appears, therefore, that the hospitalization of tuberculous patients and their treatment are not the only causes and may not even be the major causes of the changes in death rates, so that the first part of the statement by Drolet and Lowell of their importance in "modifying so favorably the mortality record" is open to question. Of equal, if not greater, importance to those concerned with the control of tuberculosis is the validity of the second part of their statement regarding failure in the preventive phase of tuberculosis campaigns.

MORBIDITY

Since accurate information regarding the incidence of tuberculosis in the general population is practically non existent, Drolet and Lowell use the number of reported cases and the ratio of deaths to cases as indices of the rate of

development of the disease. They discuss the marked decrease in the number of deaths from tuberculosis between 1930 and 1950, during which time the number of cases reported annually remained the same so that the ratio of deaths to cases ("community case fatality") decreased from 70 to 29 per cent. From this they infer that the incidence of tuberculosis has not decreased during this twenty-year period.

These data, however, do not take into consideration the number of persons examined and the percentage yield of cases of tuberculosis. While the number of cases of tuberculosis reported annually may be a reasonably accurate index of the trend of the annual incidence rate, this is true only when case-finding activities remain at the same level year after year. Any increase or decrease of these activities must result in an increase or decrease in the total number of cases found, even though these changes are not proportional to the effort ex-

TABLE 2

PER CENT YIELD OF CASES OF PULMONARY TUBERCULOSIS FOUND AMONG PERSONS EXAMINED IN UPSTATE NEW YORK CLINICS OF STATE AND LOCAL TUBERCULOSIS HOSPITALS

1939–1941 and 1948–1950

	1948–1950			1939–1941			CHANGE IN YIELD 1939–41 TO 1948–50
	Examinations	Cases	Yield	Examinations	Cases	Yield	
			per cent			*per cent*	*per cent*
All clinics	245,279	4,174	1.7	174,087	4,960	2.8	−40.4
State tuberculosis hospital clinics	76,224	838	1.1	43,814	916	2.1	−47.4
Local tuberculosis hospital clinics	169,055	3,336	2.0	130,273	4,044	3.1	−35.5

pended. In studying the trend in incidence, the percentage yield of cases of tuberculosis among those examined is more important than the total number of cases found annually. It may reasonably be assumed that, if the composition of the groups examined remains essentially unchanged and the rate of development of new cases is constant, the percentage yield of new cases should also remain constant. On the other hand, any change in percentage yield of new cases in such groups may be due to a corresponding change in the incidence rate.

For the study of the trend in incidence, data obtained from official case-finding activities in Upstate New York are used. These activities include (*1*) the examinations in clinics of tuberculosis hospitals, (*2*) community-wide mass surveys and routine roentgenographic examination of patients upon admission to general hospitals. The total number of cases reported and case rates will also be considered.

Tuberculosis hospital clinics: As shown in table 2, in spite of an increase of 40 per cent in the number of persons examined in 1948–1950, as compared with 1939–1941, there was a decrease in the number of cases reported from the clinics of state and local tuber-

culosis hospitals in Upstate New York. The percentage yield decreased 40 per cent during this time, from 2.8 to 1.7. Part of the decrease in case yield may be due to a change in the character of clinic activities during the later years to include groups of patients reporting for roentgenograms only, among whom the tuberculosis case yield is lower than among the usual type of clinic patients. However, these groups are examined in addition to the usual types of clinic patients, such as contacts and suspects, so that the decrease

TABLE 3

RESULTS OF CHEST ROENTGENOGRAPHIC EXAMINATIONS OF ADULTS EXAMINED IN MASS SURVEYS AND OF PATIENTS ADMITTED TO GENERAL HOSPITALS IN UPSTATE NEW YORK, 1948–1951

YEAR	TOTAL NUMBER EXAMINED ROENTGENOGRAPHICALLY	PROBABLY ACTIVE TUBERCULOSIS	
		Number of Cases	Rate per 1,000
Total	1,311,070	3,933	3.0
1948–1949	509,667	1,780	3.5
1950–1951	801,403	2,153	2.7

TABLE 4

NUMBER OF CASES OF PULMONARY TUBERCULOSIS REPORTED IN UPSTATE NEW YORK AND RATE PER 100,000 POPULATION

YEAR	NUMBER OF CASES	RATE PER 100,000 POPULATION	3-YEAR AVERAGE ANNUAL RATE
1935	5,733	98	—
1936	6,118	104	99
1937	5,562	94	97
1938	5,537	93	89
1939	4,765	79	83
1940	4,663	77	78
1941	4,785	78	83
1942	5,737	93	87
1943	5,532	88	86
1944	4,968	78	78
1945	4,392	69	75
1946	5,072	78	73
1947	4,831	73	75
1948	4,883	73	72
1949	4,817	70	68
1950	4,285	62	—

in the total number of reported cases, as well as the lower case yield, is evidence of a decrease in the incidence of tuberculosis in the population served by these hospitals.

Community surveys and general hospital admissions: Similar results were obtained among persons examined in community surveys and those routinely examined roentgenographically upon admission to general hospitals in Upstate New York. As shown in table 3, in spite of a 57 per cent increase in the number of examinations in 1950–1951 as compared with 1948–1949, there was an increase of only 21 per cent in the total number of cases diagnosed, while the percentage yield of cases of tuberculosis decreased 22.9 per cent.

The lower percentage yield of new cases of tuberculosis in these large segments of the Upstate population can be due to only one major factor, a decrease in the rate of development of new cases. It is also probable that this reduction in incidence is one of the important causes of the decrease in tuberculosis death rates.

Additional evidence of a decrease in the incidence of tuberculosis is obtained from a study of the total number of cases reported and the case rates for the past sixteen years in Upstate New York. As shown in table 4, although there has been some decrease in the number of reported cases, the ratio of cases per 100,000 population decreased considerably from a three-year average of 99 in 1935–1937 to 68 for 1948–1950. While part of the decrease in percentage yield of new cases of tuberculosis may be due to a larger number of persons examined in mass surveys, among whom the percentage yield is lower than among contacts, suspects, or hospital admissions, the fact that the trend over the years for the entire population is toward a lower rate, just as it is in the special population groups just mentioned, suggests a similar basic cause, a reduction in the incidence of the disease.

It is not the intent here to imply that the reduction in death rate and the downward trend in incidence have relegated tuberculosis to a status of insignificance. On the contrary, in spite of the decreasing incidence and number of deaths, it still remains high on the list of causes of illness and death, and requires continuation of all efforts for its control. Those concerned with the effect of tuberculosis upon the public health are, nevertheless, entitled to the knowledge that the disease is yielding to these efforts.

SUMMARY

In the absence of accurate information concerning the incidence of tuberculosis, the number of deaths and reported cases, as well as the ratio between the two, are used to estimate the trend of tuberculosis morbidity. Studies based upon these data suggest that the rate of development of new cases of tuberculosis has not changed during the past twenty years.

These data do not take into consideration, however, the number of persons examined and the percentage yield of new cases of tuberculosis among them. Comparative study of the results of roentgenographic examination of patients in tuberculosis hospital clinics, as well as in community chest roentgenographic surveys, and patients admitted to general hospitals of Upstate New York (New York State exclusive of New York City) show a decreasing yield in the percentage of new cases of tuberculosis. The ratio of reported cases per 100,000 population in Upstate New York has also decreased considerably since 1935. From this it is concluded that the incidence of tuberculosis is decreasing.

SUMARIO

Tendencia de la Morbidad Tuberculosa

A falta de datos exactos acerca de la incidencia de la tuberculosis, úsanse el número de defunciones y de casos comunicados, así como la proporción entre los dos, para estimar la

tendencia de la morbidad tuberculosa. Los estudios basados en dichos datos sugieren que la aparición proporcional de casos nuevos no ha variado en los últimos veinte años.

Sin embargo, esos datos no toman en cuenta el número de sujetos examinados y el porcentaje de casos nuevos de tuberculosis entre ellos. El estudio comparativo de los resultados del examen radiográfico de enfermos en las clínicas hospitalarias para tuberculosos, así como de las encuestas radiográficas colectivas, y de los enfermos admitidos en los hospitales generales del Estado de Nueva York (exclusive de la ciudad de Nueva York) revela una tendencia descendente en el porcentaje de nuevos casos de tuberculosis. La proporción de casos comunicados por 100,000 habitantes en dicha zona ha disminuído considerablemente desde 1935. De eso se deduce que va disminuyendo la incidencia de la tuberculosis.

RESUME

Tendance de la morbidité par tuberculose

En l'absence d'informations précises concernant l'incidence de la tuberculose, le nombre de décès et les cas enregistrés, ainsi que le rapport entre ces deux facteurs, sont utilisés pour évaluer l'évolution de la morbidité par tuberculose. Les études fondées sur ces données suggèrent que la proportion des cas nouveaux de tuberculose n'a pas changé depuis les dernières vingt années.

Ces données ne prennent toutefois pas en considération le nombre de sujets examinés et le pourcentage de nouveaux cas de tuberculose révélé parmi ceux-ci. Une étude comparative des résultats fournis par l'examen radiologique des malades des services cliniques de la tuberculose, ainsi que de ceux provenant des examens radiologiques pulmonaires systématiques et des services généraux des hôpitaux de l'état de New York, la ville de New York exceptée, montrent que le pourcentage de ces cas nouveaux de tuberculose est en décroissance. Le taux des cas enregistrés pour 100.000 habitants dans l'état de New York, (la ville de New York exceptée), a également diminué considérablement depuis 1935. La conclusion de ces faits est que l'incidence de la tuberculose est en décroissance.

REFERENCES

(1) Edwards, H. R., and Drolet, G. J.: Implications of changing morbidity and mortality rates from tuberculosis, Am. Rev. Tuberc., 1950, *61*, 39.

(2) Drolet, G. J., and Lowell, A. M.: Whither tuberculosis?, Dis. of Chest, 1952, *21*, 527.

THE RESPONSE TO ANTIMICROBIAL THERAPY OF ANERGIC AND PARTIALLY ANERGIC TUBERCULOUS PATIENTS[1]

I. Changes in the Tuberculin Sensitivity of Individual Patients

C. EUGENE WOODRUFF, WILBUR J. STEININGER, RUBY G. KELLY, AND HERBERT KREISEL[2]

(Received for publication August 20, 1952)

INTRODUCTION

Experimental work done with tuberculous guinea pigs after the discovery of streptomycin (1) indicated that the infection could be controlled so completely that a large proportion of the treated animals became tuberculin negative. This raised the question whether or not the same thing could be accomplished in human patients, with the human tissue being freed of tuberculous antigen to such an extent that the patient would become tuberculin negative. Such has not proved to be true. During the first two years after the introduction of streptomycin, the tuberculin sensitivity of all treated patients at the Wm. H. Maybury Sanatorium was carefully followed. In this period, 146 patients were given tuberculin tests before streptomycin therapy was started and were tested again at the conclusion of either a six-week or a ninety-day course of therapy. Most of these patients reacted to the 1:10,000 dilution of tuberculin before therapy was started, and no marked change in sensitivity was found at the end of therapy. The experience of the Veterans Administration has been similar in this respect (2). During more recent years, as confidence in the potency of streptomycin has been gained, patients who were so critically ill as to have a low level of sensitivity to tuberculin (3–6) have been treated. In most of these anergic or partially anergic patients, the change in sensitivity which followed streptomycin therapy has been dramatic. The present paper deals with the effect of streptomycin on the tuberculin sensitivity of tuberculous patients of this type.

MATERIAL AND METHODS

On admission to Maybury Sanatorium, adult patients are routinely given an intracutaneous skin test with 0.1 ml. of a 1:10,000 dilution of Old Tuberculin (OT) (0.01 mg.).[3] Tuberculin tests are observed at the end of forty-eight hours, an area of induration 5 mm. or more in diameter being considered a positive test. Patients who fail to react to the first dilution are retested at forty-eight-hour intervals with tuberculin ten times as concentrated, the final dilution used being 1:10 OT (10 mg.). Included in the study are 36 patients with sputum positive for tubercle bacilli who, on admission to the sanatorium, failed to react to the first two dilutions of tuberculin.[4] These same patients were retested with tuberculin at

[1] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

[2] Exchange Fellow from Tuberculosis Hospital, Heckeshorn, Berlin-Wannsee.

[3] For the past thirteen years tuberculin dilutions have been made from the same 2-liter bottle of OT kindly donated by Parke, Davis & Company, Detroit, Michigan.

[4] The 36 patients include *all* of the initially anergic patients who received subsequent tuberculin tests.

least once several weeks or months after the start of streptomycin therapy. This gave two or more tuberculin sensitivity reference points for each patient, making possible the construction of sensitivity curves.

In drawing the sensitivity curves, the technique which was employed in previous studies (6, 7) was used. The sensitivity levels were indicated as 0, 10, 100, 1,000, and 10,000, depending upon the dilution of tuberculin to which the patient reacted. A minimal positive reaction, i.e., a 5 mm. reaction, to 1:10,000 OT was plotted directly on the 10,000 level. Similarly, 5 mm. reactions to 1:1,000, 1:100, and 1:10 OT were plotted respectively on the 1,000, the 100, and the 10 levels. Patients who failed to react to 1:10 OT were considered to be anergic and were assigned to a 0 sensitivity level which was placed arbitrarily a short distance

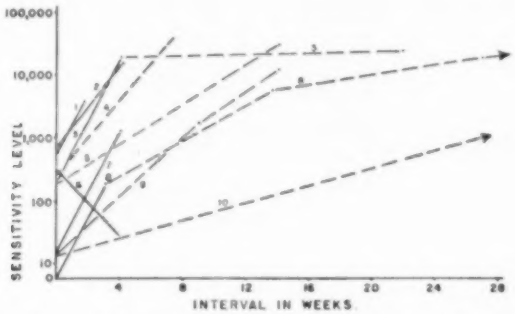


FIG. 1. Tuberculin-sensitivity curves for patients treated with streptomycin. The reference points have been connected with solid lines if the interval between readings of the tuberculin tests was four weeks or less; reference points with a time interval greater than four weeks have been connected with broken lines. Further description of curves in text.

below the 10 level (figure 1). Interpolations between the various levels were made as follows: a 10 mm. (average diameter) reaction to 1:1,000 OT was plotted one-fourth of the way up toward the 10,000 level. A 15 mm. reaction was plotted one-half of the way between the two levels, while a 20 mm. reaction was plotted three-fourths of the way up. The abscissae on the graph represent the time in weeks between the various tuberculin tests.

OBSERVATIONS

Sensitivity Curves

The data for a representative case will be given in detail, together with essential facts from the patient's history.

Case 8. P. B., an asthenic Negro girl sixteen years of age, was admitted to Wm. H. Maybury Sanatorium on June 8, 1951. The present illness began the preceding December with the appearance of "lumps" on the patient's legs. Penicillin was given by her local physician but the "lumps" were not affected. They finally broke down and discharged purulent material. In March, 1951, the patient noticed a productive cough, anorexia, and easy fatigability. She lost a total of 28 pounds in the following three months. Two days after admission to May-

bury, the temperature rose to 103.4°F. The admission roentgenogram showed dense consolidation of the right upper lobe with small areas of rarefaction at the first interspace. There were infiltrations in the left lower lobe. The sputum was positive for tubercle bacilli. Deep ulcers were found on both legs. Cultures of pus from these ulcers yielded a hemolytic, coagulase-positive *Staphylococcus* but were negative for tubercle bacilli. The patient was placed on combined streptomycin–para-aminosalicylic acid (PAS) therapy on June 15, 1951. She showed rapid clinical improvement, with healing of the ulcers on her legs; the roentgenogram showed definite clearing after three months. The chemotherapy has been continued and at the present time, more than a year after admission to the sanatorium, the prospects are favorable for surgical removal of the residual cavity.

In table 1 may be seen the data for the various sensitivity readings on the patient; her roentgenograms are shown in figures 2 through 4.

In figure 1 will be found the sensitivity curves for 10 different patients, including curve 8 constructed from the data in table 1. In this particular curve, the

TABLE 1

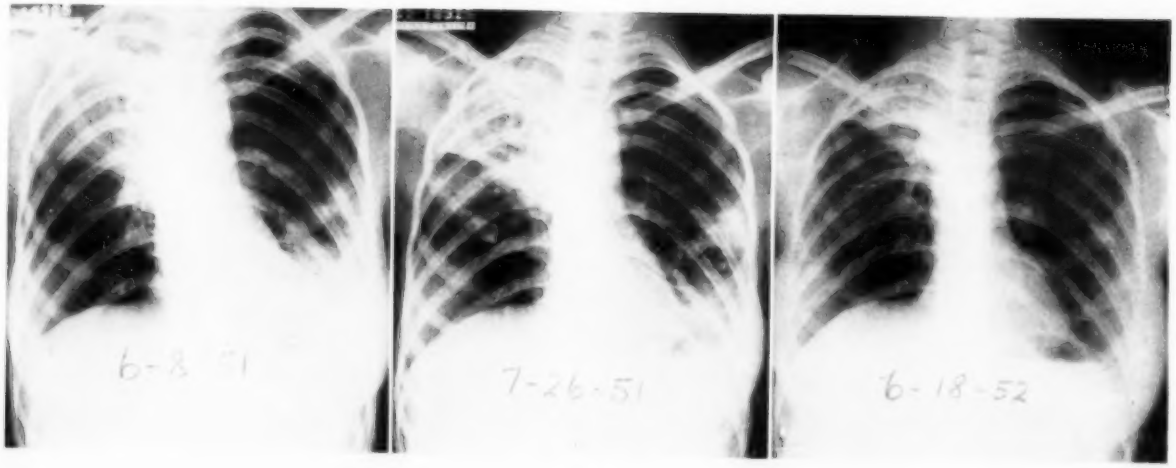
CUTANEOUS HYPERSENSITIVITY TO TUBERCULIN IN CASE 8

DATE	DILUTION OF TUBERCULIN USED	SIZE OF REACTION
6/16/1951	1:10	Negative
7/ 8/1951	1:100	10 mm.
9/13/1951	1:1,000	20 mm.
12/17/1951	1:10,000	12 mm.
7/13/1952	1:10,000	33 mm.

rapid increase in sensitivity during the first three weeks will be noted. During the following weeks the sensitivity level rose consistently but more gradually. The final reference point for the tuberculin test read on July 13, 1952, is not plotted, the arrow on the curve indicating the continued slow rise in sensitivity.

In the drawing of curve 8, as well as the nine other curves, the reference points have been connected with solid lines if the interval between readings of the tuberculin tests was four weeks or less; reference points with a time interval greater than four weeks have been connected with broken lines. In curve 10, for example, an interval of a year and one-half elapsed before the second tuberculin test was given. The arrow points toward the sensitivity level eventually reached by this patient, a 14 mm. reaction to the 1:10,000 dilution of tuberculin.

It will be noted that, with the exception of curve 6, the solid lines assume a more or less parallel direction. Curve 6 is for a patient who failed to respond well to streptomycin and who died approximately six months after his admission to the sanatorium. The parallel direction of the other solid-line curves indicates that anergic or partially anergic patients who respond favorably to streptomycin show a rapid improvement in their allergic state during the first few weeks after therapy is instituted. It is probable that a four-week skin test on Patient 10, for example, would have shown a similar abrupt increase in tuberculin sensitivity during his initial period of treatment. In most cases, after a patient reached the 10,000 level, his tuberculin sensitivity remained more or less constant. The very



Case 8

FIG. 2. Admission roentgenogram. At this time the patient failed to react to a 1:10 dilution of Old Tuberculin.

FIG. 3. Roentgenogram taken seven weeks after admission. There has been a spread of disease in the right second interspace and to the left lower lobe although the clinical condition of the patient and the level of tuberculin sensitivity have improved (table 1).

FIG. 4. Roentgenogram taken more than a year after admission. At this time the patient was highly allergic to tuberculin (table 1).

high level of sensitivity indicated by the 33 mm. reaction obtained in Case 8 (table 1) is unusual and may be associated with the color and the youth of the patient.

Of the 36 patients in the present study, all but 2 showed an eventual increase in sensitivity to the 10,000 level. The exceptions were Patient 6, already mentioned, and another patient who, at the end of two months, showed only a slight increase in sensitivity to tuberculin and who died two months later.

DISCUSSION

It is well known that in far advanced tuberculosis untreated with streptomycin, whether in man or animals, the curve showing tuberculin sensitivity declines rapidly during the last few weeks of life (6, 7). Following the successful use of streptomycin (with or without PAS) in these critically ill patients, the direction of the sensitivity curve is reversed; sensitivity to tuberculin is rapidly regained. This may be of importance in giving an indication of the prognosis in a given case. In the course of the present study it has happened repeatedly that a second tuberculin test, given at the end of four weeks, showed a markedly higher level of tuberculin sensitivity even though the accompanying roentgenogram indicated more extensive disease than when the patient was admitted. In other words, increased sensitivity to tuberculin often indicates an ultimately favorable clinical response before any favorable roentgenographic evidence can be obtained.

At the beginning of this paper, mention was made of the results obtained by Feldman, Hinshaw, and Mann (1) in their early therapeutic trials of streptomycin. It is of interest that Steenken and Pratt (8), under different experimental conditions, did not obtain a negative tuberculin reaction in guinea pigs treated with streptomycin. Indeed, the latter workers conclude that tuberculous lesions in infected guinea pigs are progressive in spite of adequate therapy "... until acquired host-resistance develops, as shown by the development of skin hypersensitiveness." The difference in the results obtained by the two groups of investigators can probably be explained by the difference in the amount of inoculum used: 0.001 mg. of tubercle bacilli in the first case and approximately 0.2 mg. in the second case. From the results of the present study on human patients, one would conclude that the clinician, also, is dependent upon changes in the responses of the host, the most readily demonstrable of which is the increase in sensitivity to tuberculin, before he can expect to see favorable roentgenographic results in anergic patients treated with streptomycin.

SUMMARY

The course of cutaneous hypersensitivity to tuberculin has been followed in 36 anergic or partially anergic tuberculous patients treated with streptomycin (with or without para-aminosalicylic acid). In every patient who responded favorably to antimicrobial therapy, the cutaneous hypersensitivity to tuberculin was found to increase. Increased hypersensitivity to tuberculin in patients of this type frequently precedes roentgenographic evidence of improvement.

SUMARIO

La Respuesta de los Tuberculosos Anérgicos y Parcialmente Anérgicos a la Terapéutica Antimicrobiana: I. Alteraciones de la Sensibilidad a la Tuberculina en Ciertos Enfermos

La evolución de la hipersensibilidad cutánea a la tuberculina fué observada en 36 tuberculosos total o parcialmente anérgicos y tratados con estreptomicina (con o sin ácido para-aminosalicílico). En todo enfermo que respondió favorablemente a la terapéutica antimicrobiana, observóse que aumentaba la hipersensibilidad cutánea a la tuberculina. El aumento de la hipersensibilidad a la tuberculina en los enfermos de este género precede frecuentemente a los signos radiográficos de mejoría.

RESUME

La réponse à la thérapie antimicrobienne chez les tuberculeux anergiques ou partiellement anergiques: I. Modifications de la sensibilité individuelle à la tuberculine

L'évolution de l'hypersensibilité cutanée à la tuberculine a été observée chez 36 tuberculeux anergiques ou partiellement anergiques traités par la streptomycine (avec ou sans PAS). Chez chacun des malades réagissant favorablement à la therapie antimicrobienne, l'hypersensibilité à la tuberculine s'est montrée acerue. L'accroissement de l'hypersensibilité à la tuberculine du malade appartenant à cette catégorie précède souvent les signes radiologiques d'amélioration.

REFERENCES

(1) Feldman, W. H., Hinshaw, H. C., and Mann, F. C.: Streptomycin in experimental tuberculosis, Am. Rev. Tuberc., 1945, *52*, 269.

(2) Barnwell, J. B., Bunn, P. A., and Walker, A. M.: The effect of streptomycin upon pulmonary tuberculosis: Preliminary report of a cooperative study of 223 patients by the Army, Navy, and Veterans Administration, Am. Rev. Tuberc., 1947, *56*, 485.

(3) Lobban, J. W.: The significance of the quantitative tuberculin reaction in the prognosis of tuberculosis, Tubercle, 1930, *12*, 19.

(4) Musacchio, F. A.: A tuberculin survey of one thousand cases of active tuberculosis, Am. Rev. Tuberc., 1940, *42*, 120.

(5) Furcolow, M. L., Hewell, B., and Nelson, W. E.: Quantitative studies on the tuberculin reaction: III. Tuberculin sensitivity in relation to active tuberculosis, Am. Rev. Tuberc., 1942, *45*, 504.

(6) Woodruff, C. E.: Tuberculin allergy in patients critically ill with tuberculosis, Am. Rev. Tuberc., 1946, *53*, 583.

(7) Woodruff, C. E., and Kelly, R. G.: The correlation between anatomical changes and the allergic state in tuberculous guinea pigs, J. Immunol., 1942, *45*, 79.

(8) Steenken, W., Jr., and Pratt, P. C.: Streptomycin in experimental tuberculosis: III. Effect on the pathogenesis of early tuberculosis in the guinea pig infected with streptomycin-sensitive H37Rv tubercle bacilli, Am. Rev. Tuberc., 1949, *59*, 664.

THE RESPONSE TO ANTIMICROBIAL THERAPY OF ANERGIC AND PARTIALLY ANERGIC TUBERCULOUS PATIENTS[1]

II. The Prolongation of Life

HERBERT KREISEL,[2] WILBUR J. STEININGER, W. LEONARD HOWARD, EDWIN E. MUELLER, AND C. EUGENE WOODRUFF

(Received for publication August 20, 1952)

INTRODUCTION

It is now generally accepted that antimicrobial therapy has a beneficial effect in the majority of cases of active tuberculosis (1). In fact, this very effectiveness is being reflected in the current increased demand for beds for tuberculous patients (2). The sanatorium bed problem is created particularly by "... those patients who would have died quickly from the disease if the improved treatment had not resulted in prolonged illness, instead of rapid death" (3). Just how many additional sanatorium beds may be needed is a matter for conjecture. It has been shown statistically that the average period of hospitalization for the tuberculous patient increased from 164 days in 1940 to 308 days in 1950 (4), but the average of 308 days obviously includes favorable cases in which the sanatorium stay was definitely shortened by chemotherapy, as well as those in which the stay was greatly prolonged. Certain it is that even patients with the most advanced pulmonary tuberculosis usually may be given a stay of their death sentences and at least be converted into "good chronics" by antimicrobial therapy. The crucial question with regard to future sanatorium needs will be answered when the proportion of these "good chronics" who can be rehabilitated and discharged into community life is determined (5).

The prognostic significance of a low level of tuberculin hypersensitivity in patients with far advanced tuberculosis is well known (6–9). Past experience at Maybury Sanatorium indicates that such patients, unless given antimicrobial therapy, usually die from the disease within a few weeks or months (10). It was thought that groups of patients with similar low levels of tuberculin hypersensitivity should be fairly closely comparable as far as life expectancy is concerned. Hence, a comparison of the length of life of such patients before and after the advent of streptomycin might add useful information concerning the impact of antimicrobial therapy on this most difficult type of sanatorium patient. With this idea in mind, a study has been made of the clinical and laboratory records of patients admitted to the Wm. H. Maybury Sanatorium during the eight-year period 1944 to 1952.

MATERIAL AND METHODS

At the present time, patients newly admitted to the adult division of the sanatorium are routinely given an intracutaneous skin test, beginning with a 1:10,000 dilution of tuber-

[1] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium) Northville, Michigan.

[2] Exchange Fellow from Tuberculosis Hospital, Heckeshorn, Berlin-Wannsee.

culin.[3] Those who fail to react to this first dilution are retested at forty-eight-hour intervals with tuberculin ten times as concentrated, the final dilution being 1:10 Old Tuberculin (OT) (10 mg.). Those patients were selected for study whose sputum was positive for tubercle bacilli, whose roentgenograms showed far advanced pulmonary disease, and who failed to show a positive reaction to both the 1:10,000 and the 1:1,000 dilutions of OT. A total of 169 of these anergic or partially anergic patients was found; 106 did not receive any antimicrobial therapy, while 63 were treated for varying lengths of time with streptomycin, either with or without para-aminosalicylic acid (PAS).[4]

The majority of the *untreated* patients in the series entered the sanatorium during the years 1944 to 1947, inclusive. Unfortunately, in these earlier years the admission routine examinations did not include a test with 1:10 OT. Necessarily then, the 169 patients are divided with regard to their allergic status into only two groups: those who showed a positive reaction to 1:100 OT (1 mg.) and those who failed to react to this amount of tuberculin. The latter group included an undetermined number of patients who were completely anergic.[5] The length of time each patient survived following his tuberculin test was determined and tabulated.

RESULTS

Table 1 (*A* and *B*) shows the survival periods by thirty-day intervals of the 106 anergic or partially anergic tuberculous patients who did not receive antimicrobial therapy. Forty-one of the patients (table 1*A*) had shown a positive reaction to the 1:100 dilution of tuberculin on admission to the sanatorium. It will be seen that 8 of these patients (19.5 per cent) survived for 120 days or longer. On the other hand, of the 65 patients who *failed* to react to 1:100 OT, only 2 (3.1 per cent) survived the 120-day period (table 1*B*).

Table 2 (*A* and *B*) shows the survival periods by thirty-day intervals of the 63 anergic or partially anergic tuberculous patients who *received* streptomycin, with or without PAS. Table 2*A* indicates that, of 44 positive reactors to 1:100 OT who received antimicrobial therapy, 36 (82 per cent) survived for 120 days or longer. Likewise, treatment with antimicrobials of the 19 patients who failed to react to 1:100 OT (table 2*B*) aided 10 (53 per cent) to survive for 120 days or longer. Data from the two tables are shown graphically in figure 1. The serious implications of the lower level of tuberculin sensitivity in both treated and untreated groups is evident.

A word of explanation is necessary concerning the tables. As will be seen in table 3, the streptomycin-treated group includes a considerably larger proportion of younger patients than is found in the untreated group. This difference in the makeup of the two groups arises largely from the fact that during 1947, 1948, and 1949, streptomycin was given only to those patients who were thought to have a good chance for eventual recovery from their disease. During these years, then,

[3] For the past thirteen years tuberculin dilutions have been made from the same two-liter bottle of OT kindly donated by Parke, Davis & Company, Detroit, Michigan.

[4] Twenty-five of the 63 patients are included in another study (11).

[5] A patient is considered to be completely anergic only if he fails to react to the 1:10 dilution of tuberculin (11).

there was a definite selection for therapy of those anergic patients with preponderantly acute exudative disease, most of whom were in the younger age groups.

Counterbalancing to some extent this factor of partial selection of patients is the fact that a large share of the treated patients received altogether inadequate chemotherapy according to present standards. Thus, more than one-third of the 63 treated patients were given streptomycin before PAS was available. Moreover, as was the custom at that time, streptomycin was given in only relatively

TABLE 1

SURVIVAL OF PATIENTS CRITICALLY ILL WITH TUBERCULOSIS (NO STREPTOMYCIN)

A. TUBERCULIN HYPERSENSITIVITY LEVEL—POSITIVE REACTION TO 1:100 OLD TUBERCULIN

TOTAL NUMBER OF PATIENTS IN GROUP	30 DAYS			60 DAYS			90 DAYS			120 DAYS		
	Deaths	Survivors		Total Deaths	Survivors		Total Deaths	Survivors		Total Deaths	Survivors	
		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
41	15	26	63.4	26	15	36.6	31	10	24.4	33	8	19.5
B. TUBERCULIN HYPERSENSITIVITY LEVEL—NO REACTION TO 1:100 OLD TUBERCULIN												
65	48	17	26.1	57	8	12.3	59	6	9.2	63	2	3.1

TABLE 2

SURVIVAL OF PATIENTS CRITICALLY ILL WITH TUBERCULOSIS (TREATED WITH STREPTOMYCIN)

A. TUBERCULIN HYPERSENSITIVITY LEVEL—POSITIVE REACTION TO 1:100 OLD TUBERCULIN

TOTAL NUMBER OF PATIENTS IN GROUP	30 DAYS			60 DAYS			90 DAYS			120 DAYS		
	Deaths	Survivors		Total Deaths	Survivors		Total Deaths	Survivors		Total Deaths	Survivors	
		Number	Per Cent		Number	Per Cent		Number	Per Cent		Number	Per Cent
44	2	42	95.5	4	40	91.0	5	39	87.7	8	36	82.0
B. TUBERCULIN HYPERSENSITIVITY LEVEL—NO REACTION TO 1:100 OLD TUBERCULIN												
19	5	14	73.8	6	13	68.6	7	12	63.4	9	10	53.0

short courses of six weeks. In the light of present knowledge it is certain that many more of the patients shown in table 2 (*A* and *B*) would have survived the 120-day period if streptomycin had not been discontinued prematurely.

Among the 169 patients were 24 Negro patients, 9 of whom received streptomycin. All of the 15 *untreated* patients died within the 120-day period, 12 of them during the first 30 days. This is in accord with past experience concerning the hopeless character of untreated far advanced tuberculosis in Negroes. Of the 9 Negro patients who received chemotherapy, 8 survived longer than 120 days, a striking tribute to the effectiveness of streptomycin.

The ages of the 169 patients included in the study range from thirteen to

eighty-four years. The data for all cases classified according to age groups are shown in table 3. In the teen age group, although the numbers are small, the 100 per cent mortality of untreated patients and the 100 per cent survival of those who received streptomycin is impressive. Doubtless, the excellent response of

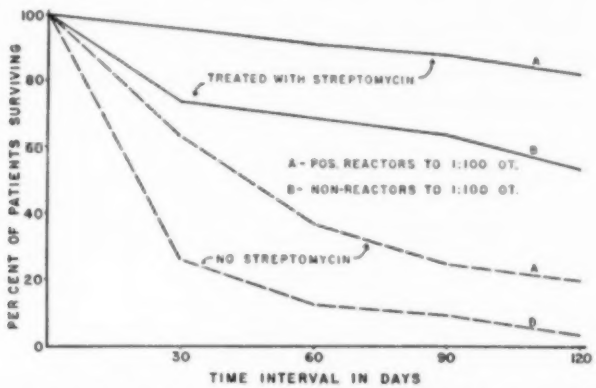


FIG. 1. The survival of patients critically ill with tuberculosis according to level of hypersensitivity and according to treatment or nontreatment with streptomycin.

TABLE 3

SURVIVAL BY AGE GROUPS OF PATIENTS CRITICALLY ILL WITH TUBERCULOSIS

	AGE GROUP	TOTAL NUMBER IN GROUP	SURVIVED 120 DAYS	
			Number	Per Cent
No streptomycin	13 to 19	4	0	0.0
	20 to 45	31	4	12.8
	46 to 84	71	6	8.5
	Total	106	10	9.5
With streptomycin	13 to 19	6	6	100.0
	20 to 45	29	22	76.0
	46 to 84	28	18	64.4
	Total	63	46	72.0

these younger patients has to do with their predominantly acute exudative type of disease (12, 13). As indicated in the table, the two other age groups also showed a satisfactory response to streptomycin.

The preceding tables leave unanswered the all-important question: What is the final status of treated and untreated patients? The fate of the 106 anergic patients who did not receive streptomycin is readily given; all of them are dead. As shown in table 4, 96 died within four months, 6 died within a year, 3 within

two years, and one within three years. The last-mentioned patient had uncontrolled diabetes when he first entered the sanatorium, and the treatment of this disease with insulin undoubtedly contributed to his long period of survival.

The current status of the 63 treated patients is shown in table 5. As indicated, there have been 31 fatalities, 29 patients died within one year, another within two years, and one within three years. Of the 6 patients with apparently arrested disease, 4 have now been discharged from the sanatorium for a period of more than three years and have experienced no reactivation of their tuberculosis. The other 2 patients have been similarly well after discharge from the sanatorium for

TABLE 4

PRESENT STATUS OF THE 106 UNTREATED ANERGIC PATIENTS

INTERVAL AFTER ADMISSION IN MONTHS	NUMBER UNDER OBSERVATION AT START OF PERIOD	DEATHS DURING INTERVAL	DISCHARGED AS APPARENTLY ARRESTED	STILL UNDER TREATMENT
0 to 4	106	96	0	10
4 to 12	10	6	0	4
12 to 24	4	3	0	1
24 to 36	1	1	0	0
	Total	106	0	0

TABLE 5

PRESENT STATUS OF THE 63 STREPTOMYCIN-TREATED ANERGIC PATIENTS

INTERVAL AFTER ADMISSION IN MONTHS	NUMBER UNDER OBSERVATION AT START OF PERIOD	DEATHS DURING INTERVAL	DISCHARGED AS APAPRENTLY ARRESTED	STILL UNDER TREATMENT
0 to 4	63	17	0	46
4 to 12	46	12	1	33
12 to 24	33	1	4	28
24 to 36	28	1	1	26
	Total	31	6	26

one and two years, respectively. The 26 patients who remain under treatment have been in the sanatorium for lengths of time which vary from 210 days to more than 4 years.

DISCUSSION

A review of the roentgenograms and clinical data of patients in the present study leaves the impression that, if today's regimen of long-term treatment with streptomycin and PAS (with or without isoniazid) had been available from the outset, a much larger proportion of these patients would have been discharged as apparently arrested cases. At the same time it should be pointed out that, had antimicrobials been given to *as large a proportion* of critically ill patients as is the case today, many more Maybury Sanatorium beds would now be occupied by chronic cases of tuberculosis. No known chemotherapeutic agent can rebuild

destroyed lung tissue, and the tuberculous patient with a major portion of his lungs destroyed may be helped temporarily by streptomycin or one of the more recent drugs only to require hospital care for the rest of his life.

Thus, standing at the bedside of a far advanced tuberculous patient with apparently hopeless disease, the physician still faces the question at times, "Is there any use in treating this patient with streptomycin?" If he has large bilateral cavities, is he liable to require hospital care for an indeterminate period of time, thereby depriving someone of a bed whose prospects for an eventual successful outcome may be much better?

Ever since streptomycin was introduced there seems to have been some reason for limiting its use. At first the antibiotic could not be obtained in quantity. Then, when the supply of streptomycin became equal to the demand, its use was proscribed in some cases because of the fear of producing resistant tubercle bacilli in the patient with a noncollapsible cavity. At the present time, the problem of drug-resistant bacilli has been appreciably lessened by the use of PAS together with reduced dosage of streptomycin. As a consequence, there is nothing but the lack of sufficient hospital beds to prevent the prompt and general use of accepted methods of treating tuberculosis. As indicated in tables 1, 2, 4, and 5, the average length of life of the most critically ill tuberculous patients has been measurably increased by antimicrobial therapy, and a not inconsiderable number of patients have been discharged as apparently arrested. With the life-saving agents presently available, no tuberculous patient should be considered a "hopeless case" without adequate trial of chemotherapy.

SUMMARY

A study has been made of the survival time of 169 anergic and partially anergic tuberculous patients, 63 of whom were treated for various lengths of time with streptomycin (with or without para-aminosalicylic acid.) Seventy-two per cent of those who received antimicrobial therapy were alive at 120 days; 51 per cent were still alive at 3 years. Of the 106 patients who did not receive antimicrobials, only 9.5 per cent survived the 120-day period, and all of them were dead at the end of 3 years.

In view of the demonstrated effectiveness of chemotherapy in these critically ill patients, no case should be considered "hopeless" without adequate trial of antimicrobials.

SUMARIO

La Respuesta de los Tuberculosos Anérgicos y Parcialmente Anérgicos a la Terapéutica Antimicrobiana: II. La Prolongación de la Vida

Este estudio versa sobre el tiempo de sobrevivencia de 169 tuberculosos total o parcialmente anérgicos, 63 de los cuales fueron tratados durante períodos variables de tiempo con estreptomicina (con o sin ácido para-aminosalicílico). Setenta y dos por ciento de los que recibieron terapéutica antimicrobiana se hallaban vivos a los 120 días; 51 por ciento todavía vivían al cabo de 3 años. De los 106 enfermos tratados sin drogas antibacterianas, sólo 9.5 por ciento sobrevivieron 120 días y todos habían fallecido al cumplirse los 3 años.

[En vista de la comprobada eficacia de la quimioterapia en esos sujetos críticamente

enfermos, no debe considerarse ningún caso como "desahuciado" sin una prueba adecuada de los medicamentos antimicrobianos.

RESUME

La réponse à la thérapie antimicrobienne des malades anergiques ou partiellement anergiques: II. La prolongation de la vie

Le temps de survivance de 169 tuberculeux anergiques ou partiellement anergiques, dont 63 avaient été traités par la streptomycine (avec ou sans PAS) pendant des périodes variées, est l'objet d'une étude. Parmi les malades soumis à la thérapie antimicrobienne, 72 pour cent étaient vivants au bout de 120 jours; 51 pour cent étaint encore vivants au bout de 3 années. Sur les 106 malades n'ayant pas reçu le traitement antimicrobien, 9.5 pour cent seulement ont dépassé la période de 120 jours; tous étaient morts à la fin des 3 années.

En raison de l'efficacité de la chimiothérapie, qui a été démontrée chez ces malades dont l'état était critique, aucun cas ne doit être considéré comme "désespéré" avant l'essai adéquat des antimicrobiens.

REFERENCES

(1) American Trudeau Society: Developments in antimicrobial therapy of tuberculosis. Am. Rev. Tuberc., 1952, *66*, 251.

(2) Cooper, D. A.: The President's Column, N. T. A. Bulletin, July, 1952, p. 113.

(3) Perkins, J. E.: Effect of isoniazid on the program of the tuberculosis association, Am. Rev. Tuberc., 1952, *66*, 615.

(4) Dempsey, M.: The tuberculosis picture today, N. T. A. Bulletin, June 1952, p. 86.

(5) American Trudeau Society: The problem of the so called "good chronic" case of pulmonary tuberculosis, Am. Rev. Tuberc., 1952, *65*, 643.

(6) Lorban, J. W.: The significance of the quantitative tuberculin reaction in the prognosis of tuberculosis, Tubercle, 1930, *12*, 19.

(7) Musacchio, F. A.: A tuberculin survey of one thousand cases of active tuberculosis, Am. Rev. Tuberc., 1940, *42*, 120.

(8) Furcolow, M. L., Hewell, B., and Nelson, W. E.: Quantitative studies on the tuberculin reaction: III. Tuberculin sensitivity in relation to active tuberculosis. Am. Rev. Tuberc., 1942, *45*, 504.

(9) Woodruff, C. E.: Tuberculin allergy in patients critically ill with tuberculosis, Am. Rev. Tuberc., 1946, *53*, 583.

(10) Woodruff, C. E., and Howard, W. L.: The importance of the tuberculin test in the differential diagnosis of pulmonary lesions, Am. Rev. Tuberc., 1951, *63*, 140.

(11) Woodruff, C. E., Steininger, W. J., Kelly, R. G., and Kreisel, H.: The response to antimicrobial therapy of anergic and partially anergic tuberculous patients: I. Changes in the tuberculin sensitivity of individual patients. Am. Rev. Tuberc., 1953, *67*, 286.

(12) Riggins, H. M., and Hinshaw, H. C.: Streptomycin—tuberculosis research project of the American Trudeau Society: A summary report. Am. Rev. Tuberc., 1949, *59*, 140.

(13) Steenken, W. Jr., Wolinsky, E., Pratt, P. C., and Smith, M.: Streptomycin in guinea pigs with discrete chronic tuberculous lesions, Am. Rev. Tuberc., 1952, *66*, 194.

ELECTROPHORETIC AND CHEMICAL SERUM PROTEIN FRACTIONS IN PULMONARY TUBERCULOSIS[1, 2]

BRUNO W. VOLK, ABRAHAM SAIFER, LINDEN E. JOHNSON, AND IRWIN ORESKES

(Received for publication September 18, 1952)

INTRODUCTION

The widespread use of mass screening by roentgenograms frequently uncovers suspected minimal lesions of tuberculosis. A more definite diagnosis of tuberculosis may then be made by culture of the tubercle bacillus. Since this is frequently a slow process requiring a waiting period of several weeks, a number of attempts have been made to obtain a more rapid diagnosis by the use of liquid media (1, 2) and by the development of a specific serologic test for tuberculosis, e.g., the hemagglutination test (3). The former procedure requires the presence of large numbers of tubercle bacilli in the patient's sputum samples; whereas the latter has been reported by various investigators (4, 5, 6) as being of little practical value as a diagnostic test.

Once the diagnosis has been definitely established, the usual bacteriologic or serologic determinations have little to offer as tests for the prognosis of the disease or as a guide to response to treatment with therapeutic agents. At present, reliance for the laboratory evaluation of the condition of the patient with active tuberculosis is dependent mainly on serial bacterial examination of sputum, roentgenographic findings, and determinations of the erythrocyte sedimentation rates. As a consequence of the inadequacies of these tests as reliable aids in following the course of this disease, many investigators have been led to study serum protein changes during various stages of pulmonary tuberculosis.

Earlier workers employed the usual salt fractionation methods, i.e., sodium sulfate or ammonium sulfate precipitation, to separate the serum total proteins into an albumin and a globulin fraction in cases of pulmonary tuberculosis (7–13). The findings showed that the total protein values tended to fluctuate around normal limits, the serum albumin tended to fall moderately, but that there was a significant increase in the globulin fraction. These studies also indicated that there was a continued rise in the globulin fraction and a fall in the albumin fraction, i.e., a decreasing A/G ratio, with increasing severity of the disease.

These reported increases in the total serum globulin led many investigators (14–17) to undertake further fractionation of tuberculosis serum into euglobulin, pseudoglobulin I, and pseudoglobulin II fractions employing the salt fractiona-

[1] From the Division of Laboratories, Jewish Sanitarium and Hospital for Chronic Diseases, Brooklyn, New York; and the U. S. Public Health Service Hospital, Manhattan Beach, Brooklyn, New York.

[2] This paper was presented before the Biological Chemistry Division of the American Chemical Society at its September, 1952, meeting in Atlantic City, New Jersey.

tion method of Howe (18). They found that the globulin increase in tuberculosis sera was due chiefly to increases in the pseudoglobulin I and euglobulin fractions.

With the advent of the electrophoretic technique of Tiselius (19, 20), it became possible to separate the serum globulins into at least four distinct fractions, i.e., alpha-1, alpha-2, beta, and gamma globulins, and to determine them *quantitatively* from their electrophoretic patterns.

Comparative studies of the electrophoretic method and Howe's salt fractionation method by many investigators (19–23) furnished conclusive evidence of the electrophoretic inhomogeneity of the Howe fractions in sera obtained from both normal and tuberculous subjects. The euglobulin fraction was found to consist mainly of electrophoretic gamma globulins but contained only approximately one-third of the gamma globulins measured electrophoretically. The pseudoglobulin I fraction was found to consist of one-half to two-thirds gamma globulin, the remainder being mostly beta globulin.

From such data it is to be expected that the major change in protein composition in tuberculosis would lie in the electrophoretic gamma globulin fraction, with possibly some changes in the beta globulin fraction. That this is true was shown in the careful electrophoretic protein studies on a large series of patients with pulmonary tuberculosis by Seibert and her associates (24). These workers found a rise in the gamma globulin serum value in minimal tuberculosis which continued to increase with the severity of the disease. In the patients with far advanced tuberculosis, they found an increase in all of the globulin fractions including the beta globulins.

While electrophoresis is a valuable method for the quantitative determination of protein fractions, its clinical use in following a large series of cases is rather limited. Electrophoretic methods require relatively large amounts of protein material, expensive equipment, and skilled technical help and permit only a few individual determinations to be performed daily. It is for this reason that many investigators (25–31) have employed protein flocculation reactions as a simple means of detecting protein changes in many disease states, including tuberculosis. These simple protein flocculation tests, while suitable for clinical studies, are *qualitative* procedures which reflect the changes in the protein patterns obtained electrophoretically. Although these tests, as usually performed, indicate primarily changes in the gamma globulin, they are also influenced by changes in the other protein fractions (32–35).

It was the nonselectivity of these protein flocculation tests for gamma globulin, as well as their qualitative nature, that led some investigators to propose the use of salt fractionation methods for the quantitative determination of gamma globulins (36–38). A *fixed concentration* of either ammonium sulfate or sodium sulfate was employed to precipitate the serum gamma globulin fraction and its protein content was then determined with the colorimetric biuret method. Svensson (39) and Majoor (40) have shown experimentally, however, that it is not possible to separate a *single* electrophoretic component from different sera by salt fractionation at *one* concentration. These findings have been confirmed, especially for disease sera, by other investigators (22, 23, 41). Still others have reported recoveries of added gamma globulin of 73 to 92 per cent, which is un-

satisfactory for a quantitative method (37, 42). Gerstl and his co-workers (43), employed the salt fractionation method of Jager and Nickerson (36) to study gamma globulin changes in a large series of pulmonary tuberculosis. The use of such semiquantitative methods for gamma globulin determinations in disease cases was criticized in the subsequent discussion by Seibert (44).

Recently a *quantitative* protein flocculation-ninhydrin method for serum gamma globulins has been developed in this laboratory (45, 46). This method checks with the electrophoretic method over a wide range of values for both normal and disease sera; uses only 0.01 to 0.02 ml. of serum per determination; recovers added gamma globulin with an average value of plus 105 per cent; and permits as many as 100 determinations to be performed daily by one technician.

In addition to providing a simple, accurate, quantitative method for the routine, clinical determination of serum gamma globulin, these studies elaborated the evaluation of a new entity, the (total serum flocculation value)/(gamma globulin flocculation value) ratio (T.S. G.G.) for both normal and disease cases. The total serum flocculation values correspond electrophoretically to the gamma globulin fraction plus a beta or beta lipoprotein fraction. The gamma globulin flocculation values correspond with the electrophoretic gamma globulin values. This ratio has been found to increase markedly in cases in which there is an increase in the beta globulin (or beta lipoproteins), e.g., nephrosis. The ratio was found to decrease in cases in which there is an increase in the gamma globulins, e.g., multiple myeloma and liver diseases.

The present paper deals with the application of these newer quantitative protein flocculation methods to a large series of pulmonary tuberculosis cases. A selected number of patients were studied periodically for more than a year in order to compare serum protein changes with changes in the clinical course of the disease. Comparative protein studies are included for each serum with the older salt fractionation methods and the newer electrophoretic technique. The diagnostic and prognostic value of serial gamma globulin determinations, and of the T.S. G.G. ratio, for cases of pulmonary tuberculosis has been confirmed by these studies.

METHODS

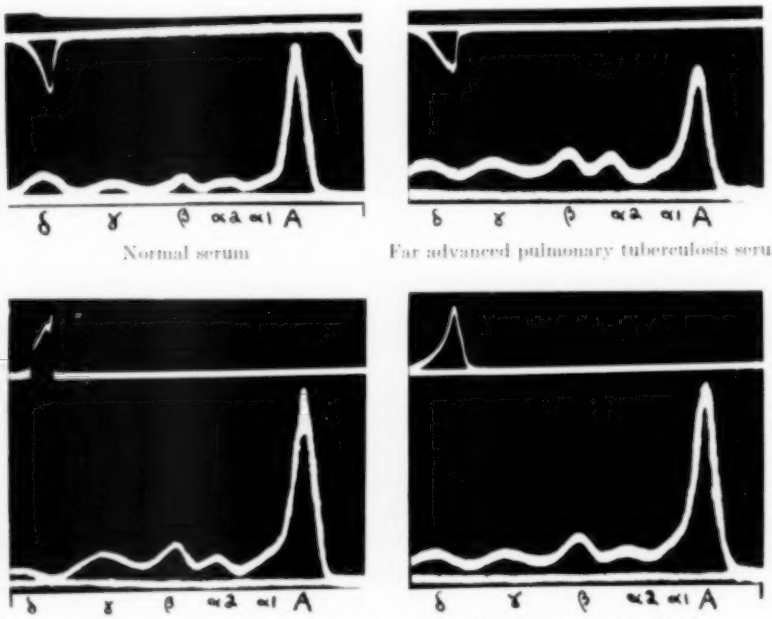
The patients studied were at the U. S. Public Health Service Hospital at Brooklyn, New York.[3] The lesions of the patients selected for study were classified as minimal, moderately advanced, or far advanced pulmonary tuberculosis in accordance with the definitions of the National Tuberculosis Association (47). None of the patients studied had any other conditions which could account for any disturbance in their protein pattern such as hepatic damage, multiple myeloma, et cetera, and other infectious diseases besides tuberculosis.

The electrophoretic and biochemical protein studies described below were performed in the laboratories of the Jewish Sanitarium and Hospital for Chronic Diseases. These laboratory results were then compared with the clinical condition of the patients evaluated independently by the medical staff of the U. S. Public

[3] Dr. Linden E. Johnson, Clinical Director.

Health Service Hospital for the same period in which the blood samples were drawn. A selected group of the patients was studied serially at approximately six-week intervals in a similar manner for approximately one year. Unless otherwise noted in the accompanying tables, all patients studied were under the standard treatment with streptomycin and para-aminosalicylic acid (PAS) (48).

Samples of blood (15 to 20 ml.) were drawn before breakfast from patients in various stages of tuberculosis and allowed to clot at room temperature. Clear, unhemolyzed serum was obtained by centrifugation at low speeds and, if not worked on immediately, was frozen



Normal serum — Far advanced pulmonary tuberculosis serum

Moderately advanced pulmonary tuberculosis serum — Arrested pulmonary tuberculosis serum

FIG. 1. Electrophoretic serum protein fractions

and kept in the freezing compartment of a refrigerator. It was found experimentally that such frozen sera could be kept up to a month with little or no change in the electrophoretic patterns.

Approximately 2 ml. of serum were required for each electrophoretic analysis. These analyses were performed by the portable Aminco-Stern electrophoresis apparatus. The optical system of the instrument is based on a modification of the original Thovert method as proposed by Philpot (49) and Svensson (50). The serum was prepared for analysis by diluting (1:3) with a barbiturate buffer (pH 8.6 to 8.7 ionic strength 0.1) and dialyzing against an excess of buffer for forty-eight hours in a refrigerator or in a mechanical dialyzer for at least two hours at 4°C. A semi-micro, clinical, double optical channel cell requiring a total volume of approximately 6 ml. was used in these experiments. Electrophoresis was carried out for approximately one hour at 15 milliamperes and 2 degrees, and the patterns were photographed and traced (Figure 1).

All calculations were done from the ascending pattern which gave better resolution and no beta anomaly. The patterns were enlarged about three and one half times, traced on paper, and the areas measured with a planimeter. The areas representing each component were defined by the method of Longsworth (51). The total protein values used to convert the percentage of total protein measured as areas into grams per 100 ml. of serum were obtained by the biuret procedure described below.

Approximately 1 ml. of serum was used for salt fractionation of the serum protein into albumin and globulin components and for the determination of the total protein value. A 26 per cent solution of sodium sulfite was used for this separation as recommended by Campbell and Hanna (52), the globulin precipitate was floated off from the albumin solution by shaking with ether and centrifugation as proposed by Kingsley (53), and the albumin and total protein content were determined colorimetrically with the biuret reagent of Gornall, Bardawill, and David (54). Albumin and total protein solutions, the protein content of which were determined with the micro-Kjeldahl method, were used as standards. Total globulin values were calculated as the difference between the total protein and the albumin value.

The photometric flocculation ninhydrin procedures used in these experiments for the *quantitative* determination of serum gamma globulins and for the total serum gamma globulin ratio values and their chemical and clinical significance have been discussed in detail in previous publications from this laboratory (45, 46).

> In brief, the method is based upon prior concentration of the serum gamma globulin fraction with minimum contamination from the other protein fractions; removal of salts by dialysis; quantitative separation of the gamma globulin from the mixture by means of a cephalin-cholesterol flocculation reaction; and the determination of the precipitated gamma globulin *quantitatively* with a colorimetric ninhydrin reaction. The entire procedure is carried out in the same tube without transfers, and blanks and standards are included with each run. The colorimetric calculations are performed in accordance with the Bouger-Lambert-Beer law. The method has been found to check with the electrophoretic method, with an average error of plus-minus 5 per cent.

RESULTS

The protein distribution in normal serum has been extensively studied, and the available data on this subject were compiled in the paper by Reiner, Feniehel, and Stern (55). Since the instrument used for the electrophoretic studies was of a design similar to that used by Reiner and her associates (55), only a small series of normal control samples were run in the present study. The results obtained checked closely with those obtained by the aforementioned investigators (55). The larger series of normal values of the electrophoretic protein components reported by Reiner, Feniehel, and Stern (55) were used as a guide in determining deviations from the normal patterns and in performing the statistical calculations reported in the present paper. For comparative purposes, the statistical data shown at the bottom of each table were calculated in the same manner as given in Seibert's paper (24).

Sera from patients with minimal tuberculosis: The protein studies for 21 cases of minimal tuberculosis are given in table 1. Two of these (Cases 119 and 122) are listed as minimal *active* cases and are not included in the statistical evaluation

TABLE 1

MINIMAL PULMONARY TUBERCULOSIS

CASE NUMBER	DATE	ELECTROPHORETIC DATA								FLOCCULATION DATA			CHEMICAL DATA			
		Albumin	Globulin	Alpha$_1$	Alpha$_2$	Beta	Gamma	A/G	Beta + Gamma / Gamma	Total Serum = Gamma Globulin	Globulin Clot = Gamma Globulin	T.S./G.G. Ratio	Total Protein	Albumin	Globulin	A/G
Active cases																
119	11/29/51	3.29	3.76	0.53	0.69	1.05	1.49	0.88	1.70	2.16	1.51	1.43	7.05	—	—	—
122	11/30/51	2.88	3.26	0.42	0.61	1.31	1.28	0.80	2.03	2.35	1.43	1.64	6.50	3.80	2.70	1.41
Arrested cases																
129	12/10/51	3.91	3.06	0.52	0.88	1.04	0.63	1.28	2.65	1.40	1.06	1.32	6.97	5.20	1.77	2.94
130	12/10/51	4.15	3.53	0.48	0.71	1.02	1.32	1.17	1.77	—	—	—	7.68	5.20	2.48	2.09
131	12/10/51	3.70	3.40	0.50	0.73	1.17	0.99	1.09	2.18	2.05	1.34	1.53	7.09	5.04	2.05	2.46
132	12/10/51	3.95	3.73	0.51	0.96	1.23	1.04	1.06	2.18	1.72	1.22	1.41	7.68	5.04	2.64	1.91
133	12/10/51	3.80	3.29	0.51	0.69	1.27	0.91	1.16	2.41	1.87	1.25	1.50	7.09	4.84	2.25	2.15
140	1/3/51	4.13	3.03	0.46	0.86	0.86	0.85	1.36	2.02	1.61	0.90	1.79	7.16	4.57	2.59	1.76
141	1/3/51	3.53	2.79	0.46	0.65	1.09	0.59	1.27	2.95	1.38	0.62	2.22	6.32	4.15	2.17	1.91
142	1/3/51	3.96	3.14	0.42	0.59	1.02	1.11	1.26	1.92	1.58	0.82	1.93	7.10	4.26	2.84	1.50
144	1/10/52	4.05	3.79	0.46	0.83	1.33	1.17	1.07	2.14	2.11	1.13	1.87	7.84	5.10	2.74	1.86
145	1/18/52	4.69	3.19	0.38	0.67	1.19	0.95	1.47	2.25	1.86	1.14	1.63	7.88	5.42	2.46	2.20
146	1/14/52	3.31	4.01	0.55	0.81	1.53	1.13	0.83	2.36	1.84	1.11	1.65	7.32	4.60	2.72	1.69
147	1/14/52	3.83	3.67	0.50	0.95	1.08	1.13	1.04	1.94	1.82	1.18	1.54	7.50	5.15	2.35	2.19
148	1/11/52	3.45	3.54	0.48	0.91	1.27	0.88	0.97	2.45	1.74	1.10	1.58	6.99	4.48	2.51	1.78
149	1/11/52	4.10	4.10	0.52	0.72	1.43	1.44	1.00	1.99	2.00	1.37	1.46	8.20	5.20	3.00	1.73
150	1/11/52	4.74	3.80	0.47	0.72	1.32	1.29	1.25	2.03	2.00	0.91	2.20	8.54	5.38	3.16	1.70
151	1/15/52	4.82	3.26	0.44	0.65	1.01	1.16	1.48	1.87	1.72	1.11	1.55	8.08	5.76	2.32	2.48
152	1/15/52	4.23	3.09	0.38	0.72	0.90	1.10	1.37	1.82	1.93	1.15	1.68	7.32	5.54	1.78	3.11
153	1/11/52	3.72	2.90	0.32	0.60	1.19	0.79	1.28	2.50	1.44	1.12	1.29	6.62	5.26	1.36	3.87
154	1/12/52	3.81	3.51	0.59	0.66	1.13	1.13	1.08	1.99	1.88	1.19	1.57	7.32	5.71	1.61	3.55
Arithmetic mean (arrested only)		4.00	3.41	0.47	0.75	1.17	1.03	1.18	2.18	1.77	1.09	1.65	7.40	5.05	2.36	2.26
Standard deviation corrected		0.41	0.37	0.06	0.12	0.17	0.22	0.17	0.31	0.22	0.18	0.26	0.54	0.46	0.48	0.66
Significance ratio		1.4	—	2.5	7.0	7.7	0.8	4.7	—	—	—	—	1.9	—	—	—

for this group. These 2 cases represent hospital employees who were discovered in the very early stage of the disease to have contracted tuberculosis. These 2 patients with minimal active tuberculosis were the only ones available for the protein studies as this U. S. Public Health Service Hospital chiefly admits patients with moderately and far advanced disease.

In agreement with Seibert's findings (21) on a much larger series of such cases, the most characteristic change in the sera of patients with minimal tuberculosis is a distinctly elevated gamma globulin and a decreased electrophoretic albumin value. The other components remain relatively normal.

The remainder of the group consisted of a series of 19 patients with minimal arrested tuberculosis. These patients had been discharged from the hospital and were examined in the outpatient clinic at three-month intervals. In all cases clinical and laboratory findings were negative with respect to the activity of the tuberculous lesions. In contrast to the findings in minimal *active* tuberculosis, the gamma globulin concentration in this "inactive" group was usually within normal limits. Nevertheless, the alpha-2 and beta fractions showed significant increases for the group above normal limits, and the electrophoretic A/G ratio was somewhat decreased.

From the protein flocculation data for the minimal arrested group, it can be observed that the mean "flocculation" gamma globulin value remained within normal limits. The mean T.S./G.G. ratio value was somewhat increased as was the electrophoretic (beta plus gamma)/gamma ratio. This would appear to reflect changes in the beta globulin fraction, which was markedly elevated above normal for this group. The chemical protein data obtained with the salt fractionation procedure showed no significant changes from the normal values for this group.

Sera from patients with moderately advanced tuberculosis: The results obtained for the protein studies in 14 cases of moderately advanced pulmonary tuberculosis are given in table 2. When the electrophoretic values were compared to normal serum components, there was a marked decrease in the albumin fraction, an increase in the alpha-2 and beta globulins, and a correspondingly large decrease in the electrophoretic A/G ratio. When the values from this group were compared with those obtained in the minimal arrested group, the most marked changes noted were a decrease in the albumin fraction, an increase in the gamma globulin fraction, and a marked decrease in the electrophoretic A/G ratio. For the flocculation results, there was an increase in the "flocculation" gamma globulin value. The T.S./G.G. ratio decreased slightly as did the electrophoretic (beta plus gamma)/gamma ratio. The chemical protein values for this group, as compared with those for the arrested cases, showed a somewhat decreased (but normal) total protein value, a decreased albumin fraction together with an increased globulin fraction, which resulted in a further decreased A/G ratio.

Sera from patients with far advanced tuberculosis: The protein studies for 17 patients with far advanced pulmonary tuberculosis are given in table 3. For the electrophoretic data, as compared with normal sera, statistically significant variations were obtained for all the components. As compared with the moder-

TABLE 2

MODERATELY ADVANCED PULMONARY TUBERCULOSIS

CASE NUMBER	DATE	ELECTROPHORETIC DATA								FLOCCULATION DATA			CHEMICAL DATA			
		Albumin	Globulin	Alpha$_1$	Alpha$_2$	Beta	Gamma	A/G	Beta + Gamma / Gamma	Total Serum = Gamma Globulin	Globulin Clot = Gamma Globulin	T.S./G.G. Ratio	Total Protein	Albumin	Globulin	A/G
109	11/18/51	3.44	4.02	0.63	0.66	1.48	1.25	0.88	2.18	1.96	1.23	1.60	7.56	4.39	3.07	1.46
110	11/18/51	3.52	3.25	0.82	0.63	1.13	0.76	1.09	2.38	1.90	1.00	1.90	6.76	4.59	2.17	2.11
111	11/18/51	2.63	4.54	0.60	1.32	1.01	1.61	0.58	1.63	2.17	1.39	1.56	7.17	3.52	3.65	0.96
112	11/18/51	3.87	3.47	0.47	0.83	1.06	1.10	1.11	1.95	1.71	0.92	1.85	7.34	4.87	2.47	1.97
114	11/26/51	2.97	3.75	0.54	0.90	0.95	1.36	0.79	1.69	1.83	1.22	1.50	6.72	4.42	2.30	1.92
116	11/26/51	3.60	3.90	0.62	0.61	1.08	1.29	1.00	1.84	1.73	1.23	1.41	7.22	4.75	2.47	1.92
117	11/26/51	3.02	3.86	0.48	0.78	1.37	1.27	0.79	2.08	1.73	1.23	1.41	6.88	4.42	2.46	1.80
118	11/26/51	3.15	3.01	0.56	0.72	1.04	0.69	1.04	2.52	1.40	0.93	1.51	6.16	4.23	1.93	2.19
121	11/29/51	3.73	2.88	0.36	0.58	1.18	0.76	1.29	2.52	2.05	1.13	1.81	6.61	3.80	2.81	1.35
123	11/29/51	3.63	3.53	0.44	0.60	1.23	1.26	1.03	1.97	2.34	1.35	1.74	7.16	4.27	2.89	1.48
125	11/29/51	2.90	3.61	0.54	0.81	1.40	0.94	0.80	2.50	1.81	1.10	1.65	6.51	4.27	2.24	1.90
126	11/29/51	3.66	3.22	0.43	0.50	0.92	1.37	1.14	1.67	2.14	1.42	1.50	6.88	4.51	2.37	1.90
127	11/29/51	3.34	3.82	0.62	0.83	1.08	1.29	0.88	1.84	1.87	1.32	1.42	7.16	4.36	2.80	1.56
155	11/25/51	3.92	3.25	0.50	0.75	1.00	1.00	1.21	2.00	1.71	1.27	1.35	7.17	4.53	2.61	1.71
Arithmetic mean		3.39	3.56	0.54	0.76	1.13	1.13	0.97	2.06	1.88	1.19	1.59	6.95	4.36	2.59	1.73
Standard deviation corrected		0.39	0.43	0.11	0.19	0.17	0.26	0.17	0.32	0.24	0.16	0.18	0.37	0.32	0.43	0.33
Significance ratio		11.0	—	1.9	4.5	6.0	2.6	12.0	—	—	—	—	3.9	—	—	—

TABLE 3

FAR ADVANCED PULMONARY TUBERCULOSIS

CASE NUMBER	DATE	ELECTROPHORETIC DATA							FLOCCULATION DATA				CHEMICAL DATA			
		Albumin	Globulin	Alpha$_1$	Alpha$_2$	Beta	Gamma	A/G	Beta + Gamma / Gamma	Total Serum + Gamma Globulin	Globulin Clot + Gamma Globulin	T.S./G.G. Ratio	Total Protein	Albumin	Globulin	A/G
88	11/1/51	2.44	4.91	0.91	0.99	1.47	1.55	0.50	1.96	2.51	1.71	1.47	7.35	4.37	2.98	1.47
89	11/1/51	2.30	4.28	0.51	1.11	1.42	1.24	0.53	2.14	2.33	1.52	1.53	6.58	3.89	2.69	1.44
90	11/1/51	2.92	4.86	0.55	0.88	1.29	2.14	0.60	1.60	3.01	2.09	1.44	7.77	4.37	3.40	1.29
96	11/5/51	2.52	5.22	0.67	1.04	1.39	2.11	0.48	1.66	3.10	1.87	1.66	7.74	2.85	4.89	0.58
97*	11/5/51	2.91	4.25	0.55	0.99	1.19	1.52	0.68	1.78	2.19	1.58	1.39	7.16	3.83	3.33	1.15
98	11/5/51	3.14	3.61	0.49	0.78	1.16	1.18	0.87	1.99	2.05	1.18	1.73	7.65	3.95	3.70	1.07
99	11/5/51	3.10	3.30	0.45	0.88	1.18	0.80	0.93	2.47	1.63	0.96	1.70	6.40	4.30	2.10	2.04
100	11/5/51	1.59	4.71	0.69	1.00	0.84	2.18	0.34	1.39	2.23	1.73	1.28	6.30	2.41	3.89	0.62
101	11/5/51	2.29	4.29	0.49	1.23	1.45	1.12	0.54	2.15	2.07	1.54	1.34	6.58	3.44	3.14	1.09
120	12/3/51	2.76	3.35	0.47	0.70	0.91	1.26	0.83	1.72	2.27	1.27	1.79	6.11	3.80	2.31	1.64
124	12/7/51	3.47	4.24	0.41	0.88	1.53	1.43	0.82	2.07	2.19	1.51	1.45	7.71	4.41	3.30	1.33
128	12/10/51	3.88	4.86	0.52	0.68	1.28	2.38	0.80	1.54	—	—	—	8.74	4.69	4.05	1.16
135	12/10/51	3.09	4.40	0.51	0.86	1.21	1.82	0.70	1.67	2.36	1.81	1.30	7.49	4.00	3.49	1.15
136	12/18/51	2.07	5.36	0.69	0.86	1.55	2.27	0.39	1.68	2.91	2.36	1.23	7.43	2.85	4.38	0.65
137	12/18/51	2.91	4.52	0.50	1.06	1.48	1.48	0.65	2.00	2.41	1.64	1.47	7.43	3.75	3.68	1.02
138	12/27/51	2.46	5.26	0.81	1.14	1.48	1.82	0.47	1.81	2.63	1.90	1.38	7.72	3.76	3.96	0.95
139	12/27/51	1.42	4.38	0.60	1.02	1.68	1.08	0.32	2.56	1.87	1.02	1.83	5.80	2.41	3.39	0.71
Arithmetic mean		2.66	4.46	0.58	0.95	1.33	1.61	0.62	1.89	2.36	1.61	1.50	7.17	3.72	3.45	1.14
Standard deviation corrected		0.63	0.62	0.13	0.14	0.22	0.48	0.19	0.34	0.40	0.40	0.19	0.76	0.69	0.70	0.39
Significance ratio		13.5	—	3.8	13.2	9.8	7.6	20.4	—	—	—	—	0.5	—	—	—

* This patient also has carcinoma of the prostate with metastases to bone.

TABLE 4

PERIODIC STUDIES OF SELECTED PULMONARY TUBERCULOSIS CASES WITH THE PROTEIN FLOCCULATION METHOD

CASE NUMBER	DATE	TOTAL SERUM = GAMMA GLOBULIN	GLOBULIN CLOT = GAMMA GLOBULIN	T.S./G.G. RATIO	CLASSIFICATION	ROENTGENOGRAPHIC CHANGES	CLINICAL PROGRESS
			(a) Terminal cases				
		gm. per 100 ml.	*gm. per 100 ml.*				
48	7/10/51	2.94	2.13	1.38	F.A.	Progressive caseation and cavitation bilaterally	Died 8/14/51 of pulmonary tuberculosis
	8/ 9/51	2.29	1.87	1.22	Active		
35	7/ 2/51	2.96	2.16	1.37	M.A.	Nodular infiltration throughout both lung fields	Died 8/20/51 of tuberculous meningitis
	8/ 9/51	2.22	1.80	1.24	Active		
31	6/27/51	3.23	2.48	1.30	F.A.	Cavitary tuberculosis of right upper lobe with implantation to both lungs	Progressive caseation (*see* figure 2)
	9/10/51	3.29	2.70	1.22	Active		
	11/ 1/51	2.94	2.46	1.19	Bilateral		
49	7/10/51	3.21	2.65	1.21	F.A.	Progressive cavitation	Progressive caseocavernous tuberculosis; died 3/7/52
	8/ 4/51	2.53	1.98	1.28	Active		
	11/ 1/51	3.01	2.09	1.44			
86	10/ 1/51	2.14	1.77	1.21	F.A.	Progressive cavitation	Progressive downhill course; died 4/1/52
	12/ 5/51	2.23	1.73	1.28	Active		
37	7/ 2/51	3.23	2.23	1.45	F.A.	Progressive cavitation and caseation	Progressive downhill course; died 1/22/52
	8/ 4/51	3.34	2.20	1.52	Active		
	1/ 2/52	2.41	1.64	1.47	Chronic		
			(b) Stabilized cases				
16	6/15/51	2.06	1.56	1.32	F.A.	Roentgenograms show no change; bilateral cavitation	Disease essentially stabilized
	10/26/51	2.41	1.61	1.50	Active		
	11/ 1/51	2.51	1.71	1.47	Critical		
42	7/ 6/51	1.58	1.29	1.22	F.A.	Bilateral fibrocaverous tuberculosis	Stabilized and improved clinically (*see* figure 3)
	9/10/51	2.36	1.50	1.57	Active		
	11/ 6/51	2.19	1.58	1.39	Chronic		
51	7/16/51	2.27	1.66	1.37	F.A.	Bilateral fibrocavernous tuberculosis with progressive improvement	Improving gradually
	8/ 9/51	2.15	1.66	1.29	Active		
	1/2/52	2.63	1.90	1.38			

TABLE 4—*Concluded*

CASE NUMBER	DATE	TOTAL SERUM GAMMA GLOBULIN	FLOCCULATION GLOBULIN CLOT = GAMMA GLOBULIN	T.S./G.G. RATIO	CLASSIFICATION	ROENTGENOGRAPHIC CHANGES	CLINICAL PROGRESS
		gm. per 100 ml.	*gm. per 100 ml.*				
(c) Cases showing improvement							
10	5/14/51	2.12	2.05	1.03	M.A.	Gradual resolution and contraction of area of infiltration	Progressive improvement; right upper lobectomy 1/3/52; uneventful convalescence (*see* figure 4)
	9/10/51	3.24	2.54	1.28	Active		
	12/ 5/51	1.87	1.32	1.42			
33	7/ 2/51	2.02	1.60	1.26	F.A.	Slight improvement	Good condition clinically; gaining weight
	11/ 6/51	1.63	0.96	1.70	Active		
	12/17/51	1.70	1.11	1.53	Chronic		
50	7/10/51	2.74	1.84	1.49	F.A.	Gradual resolution of infiltrated areas with fibrosis	Case clinically stabilized
	9/10/51	2.25	1.60	1.41	Active		
	1/ 2/52	1.87	1.02	1.83			
55	7/16/51	2.65	2.14	1.24	M.A.	Gradual resolution of infiltrated areas	Improved; patient discharged
	10/ 2/51	2.24	1.81	1.24	Active		
	12/11/51	2.14	1.42	1.50			
(d) Cases showing decline							
39	7/ 6/51	2.16	1.34	1.61	F.A.	Bilateral large cavitation	Gradual downhill course
	10/ 1/51	2.33	1.52	1.54	Active		
	11/ 6/51	2.07	1.54	1.34	Chronic		
46	7/10/51	2.37	1.94	1.22	F.A.	Progressive caseation and cavitation	Gradual downhill course, severe cough
	8/ 9/51	2.19	1.69	1.29	Active		
	12/ 9/51	2.91	2.36	1.23			

F.A. = far advanced pulmonary tuberculosis.
M.A. = moderately advanced pulmonary tuberculosis.

ately advanced group, the significant changes were a markedly decreased albumin and electrophoretic A/G ratio, markedly increased alpha-2 and gamma globulins, and slightly increased alpha-1 and beta globulins. The protein flocculation results for the far advanced cases, as compared with those for the moderately advanced group, showed a marked increase in the "flocculation" gamma globulin value and a decrease in the T.S./G.G. ratio as well as a decrease in the electrophoretic (beta plus gamma)/gamma ratio. The chemical protein fractions for this group, as compared with those for the moderately advanced cases, showed a

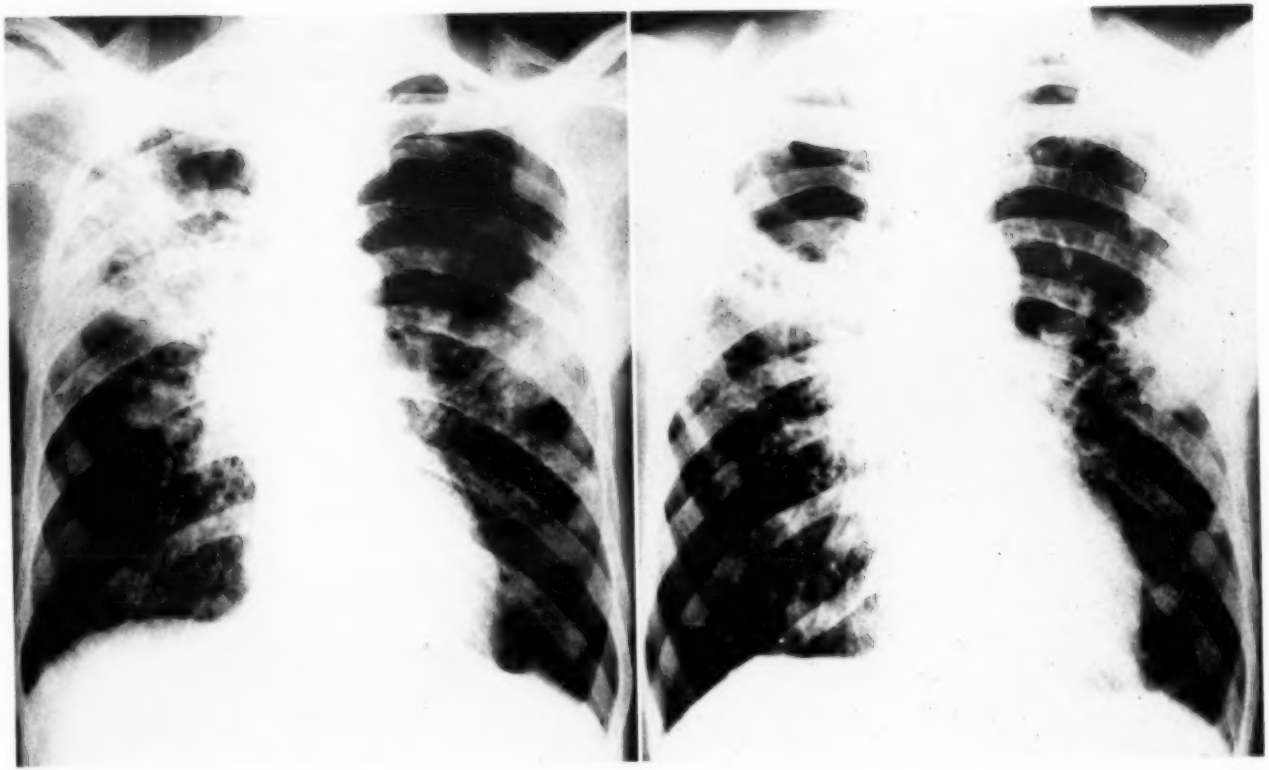


FIG. 2. Terminal case

(Left) Roentgenogram taken on December 21, 1950. Cavitary tuberculosis of the right upper lobe with implantation in the right middle and right lower lobes and spread to the contralateral left side.

(Right) Roentgenogram taken on January 7, 1952. The right upper lobe has become completely excavated. There is some clearing of the tuberculous process on the left side.

FIG. 3. Stabilized case

(Left) Roentgenogram taken on May 3, 1951. Cavitary tuberculosis of the right upper lobe. Evidence of bilateral spread with mostly fibrotic and calcific infiltration. Contraction of the right upper lobe.

(Right) Roentgenogram taken on February 7, 1952. No change has taken place.

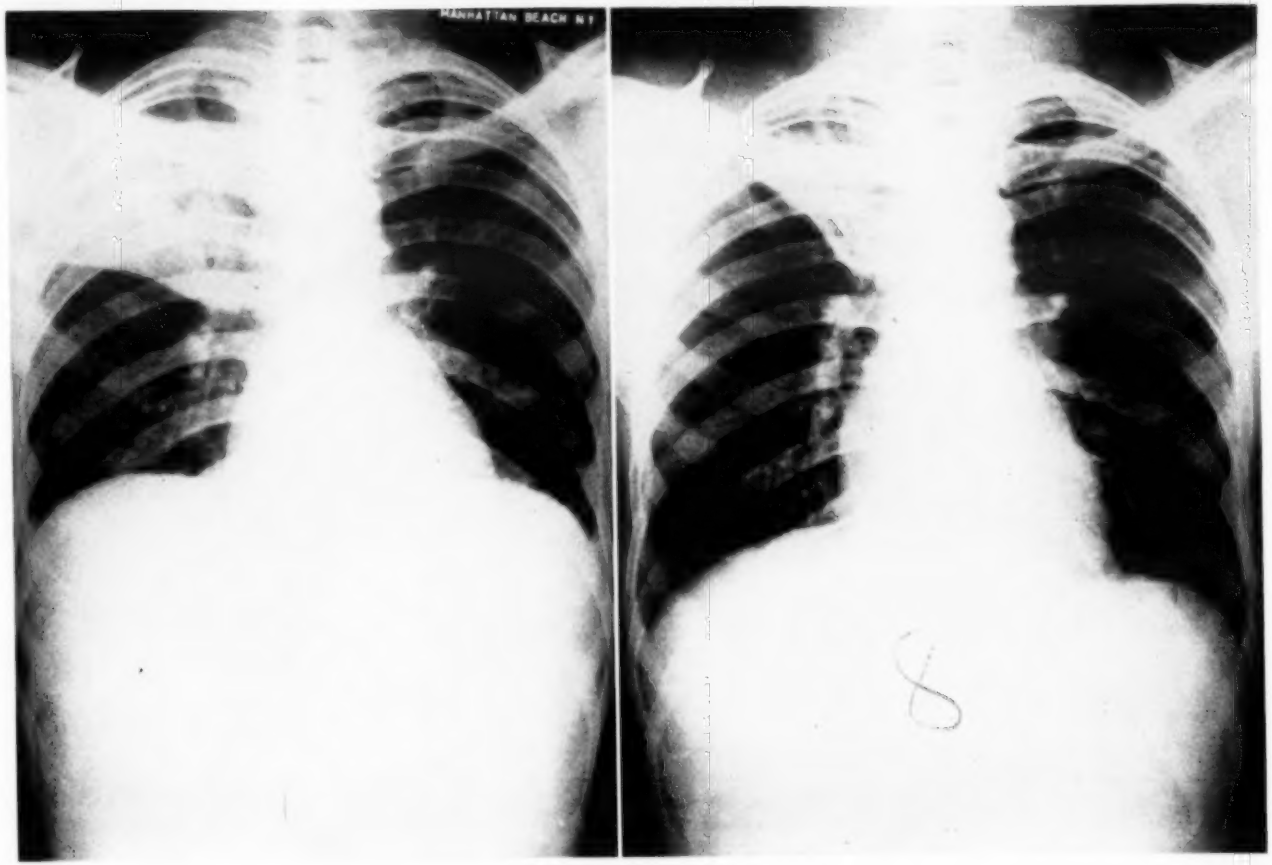


FIG. 4. Improved case

(Left) Roentgenogram taken on May 8, 1951. Right upper lobe cavity with atelectasis.
(Right) Roentgenogram taken on December 28, 1951. There is contraction of the right upper lobe. The cavity is no longer clearly seen.

markedly increased globulin fraction and a markedly decreased albumin and A/G ratio. The total protein value showed a slight rise for this group which was the result of the effect of the large increase in the globulin fraction.

Periodic study of 15 patients with pulmonary tuberculosis: Fifteen patients with pulmonary tuberculosis were selected for periodic study with the protein flocculation procedures as these methods required only small amounts of blood. These cases were selected for the following reasons:

Terminal cases: to show the nature of the protein changes just prior to death.
Stabilized cases: in which little or no change in the clinical condition of the patient occurred during the period of this study.
Labile cases: in which there was an obvious improvement or decline, respectively, in the clinical condition of the patient during the period of this study.

A comparison of the laboratory results obtained in the periodic studies of serum protein changes of these individual tuberculous patients with their clinical status is given in table 4. These data may be summarized as follows:

In terminal cases, rather high "flocculation" gamma globulin values were obtained, i.e., more than 1.7 gm. per 100 ml., together with T.S./G.G. ratios of less than 1.40. Although in some instances the "flocculation" gamma globulin value decreased somewhat just prior to death, the T.S./G.G. ratio generally decreased for this group.

In stabilized cases in the far advanced category, there were increased "flocculation" gamma globulin values, i.e., approximately 1.6 gm. per 100 ml., and a mean T.S./G.G. ratio of approximately 1.40. Periodic determinations of both of these quantities showed relatively little change during a period of approximately one year.

In cases in which improvement of the disease occurred, the "flocculation" gamma globulin values decreased and the T.S./G.G. ratio values increased during the period of study, i.e., both sets of values tended to revert back to the range of normal serum.

In cases in which the tuberculosis was progressing, there were increased "flocculation" gamma globulin values and decreased T.S./G.G. ratio values during the time of study.

In addition to the clinical data incorporated in table 4, a pair of roentgenograms for a typical case in each of the above groups is presented in figures 2 to 4. Each set represents one chest roentgenogram obtained in the early part of the periodic study and a second film obtained later during the study.

DISCUSSION

In general, the electrophoretic results for protein distribution for various stages of pulmonary tuberculosis obtained in the present study confirm those of Seibert and her co-workers (24). The major discrepancies between the two series lie in the values obtained for alpha-2 and gamma globulins in moderately advanced tuberculosis, for which Seibert reports considerably higher values. This may simply be due to the random distribution of the cases grouped into this

category at the time each study was made. For example, the moderately advanced cases in the present series may have contained a large number of lesions which were responding to treatment (PAS plus streptomycin) and were soon to become arrested. In Seibert's group there may have been a larger number of lesions which were actively progressive. Since Seibert's study is purely statistical and contains no data on the individual cases or any periodic studies, it is not possible to resolve the discrepancies between her results and those of the present study.

Better agreement between the two studies can be observed in the observations on far advanced tuberculosis, in which in both series significant increases in the alpha-2, beta, and gamma globulins, and a marked decrease in the albumin fractions were found. Another disease category in which there is good correlation, except for the finding of somewhat increased beta globulins in the present study, is in the minimal arrested group.

Another finding of clinical importance from a diagnostic viewpoint in Seibert's paper is the rather marked increase in serum gamma globulin values in minimal *active* cases. The present writers have confirmed this finding in several cases of employees who developed tuberculosis (Cases 119 and 120), as previously stated. However, the importance of increased gamma globulin values as a diagnostic test has been confirmed in another way. Two patients (Cases 128 and 150) believed to have arrested disease on clinical grounds were found to have increased gamma globulin values on routine checking. On further evaluation of their clinical status, they were discovered to have active tuberculosis which necessitated their rehospitalization. Seibert and her associates (24) have postulated that such a rise in gamma globulin in early tuberculosis is an antibody response. It should be noted that such a response resulting in an increased gamma globulin is a common characteristic of many hepatic and infectious diseases (56), and such conditions should be excluded before making the diagnosis of early or recurrent tuberculosis. Several other patients with somewhat increased gamma globulin values, e.g., Cases 130 and 149, are probably in this category exhibiting no clinical signs of recurrent tuberculosis. The remainder of the 19 patients were normal, both with respect to their electrophoretic patterns and their clinical evaluation as arrested tuberculosis cases.

Both from the present study and Seibert's findings, it is evident that the electrophoretic A/G ratio is one of the best indicators of the general condition of a patient with tuberculosis and is a valuable prognostic tool. The significance ratio, calculated according to Gavett (57), is greater for the A/G quotient than for any other electrophoretic component for the various groups studied. The present investigation provides the additional information that the ordinary chemical A/G ratio, as performed with the procedures previously described, is also a useful method for following changes in the condition of the patient, as has been repeatedly stated by previous investigators (10–13). In fact this simple and rapid chemical procedure yields A/G values *which on a statistical basis* give a constant 2:1 ratio in comparison with the electrophoretic A/G ratio. It should be emphasized that such a relationship represents only a *statistical* average and applies to but a few individual cases, as can be seen from the data for all the

patients as given in tables 1 through 3. The results obtained for the chemical A/G ratios in these studies are not strictly comparable to those reported in the older literature mentioned previously in that a 26 per cent sodium sulfite solution was used to precipitate the globulins in place of the usual 21.5 per cent sodium sulfate used in the Howe procedure. The filtrate obtained from these higher salt concentrations has been reported by some writers as giving albumin values in better agreement with the electrophoretic albumin (40, 58). However, the present writers have found electrophoretic albumin values which are approximately 1.0 gm. per 100 ml. lower on the average, compared with the chemical values for tuberculosis sera.

In previous papers (45, 46) dealing with quantitative protein flocculation methods, the "flocculation" gamma globulin value was found to correspond very closely to the electrophoretic gamma globulin over a wide range of concentrations. This agreement can be seen both from the mean values listed at the bottom of each table and from the data on the individual cases. It should also be noted that the standard deviation from the mean for the gamma globulin concentration is less for the protein flocculation method than for the electrophoretic procedure. The range of "flocculation" gamma globulin values for normal sera is 0.72 to 1.25 gm. per 100 ml., with a mean value of 1.00.

From these previously published chemical and electrophoretic studies, it has been postulated that the total serum-gamma globulin value listed in these tables constitutes all of the electrophoretic gamma globulin plus a variable fraction of the electrophoretic beta globulin. When this total serum-gamma globulin value is divided by the "flocculation" gamma globulin concentration for each serum, a ratio is obtained which for normal sera has a range of 1.40 to 1.83, with a mean value of 1.55. Examination of the mean values for this ratio in tables 1 through 3 shows that it decreases with the severity of the disease but that it falls below the normal value only for the patients with far advanced disease. This decrease in the mean value of the ratio with the advanced stage of the disease closely parallels that of the electrophoretic (beta plus gamma)/gamma ratio for each series of cases. However, the more important significance of this T.S./G.G. ratio is seen in the data presented in table 4, in which a series of selected cases was run serially with the flocculation procedures. In table 4 (*a*) data are presented for a series of 6 terminally ill patients with far advanced pulmonary tuberculosis, all of whom died during the course of this study. Such patients exhibit a gamma globulin value well above the mean for this group, i.e., more than 1.60 gm. per 100 ml., but, just as important, a T.S./G.G. ratio of less than 1.30. Persistent findings of such a nature in a case of pulmonary tuberculosis over a period of time constitute good objective evidence for the rapid advance of the disease and its probable terminal nature.

This viewpoint is further substantiated by the fact that, of the approximately 100 patients studied with the quantitative protein flocculation methods, only 7 died during the course of the present study from *any* cause, including tuberculosis. Although in Case 37 (table 4) the T.S./G.G. ratio was more than 1.40, the "flocculation" gamma globulin value was so markedly elevated above most other cases that it was assigned to this group on the basis of this biochem-

ical finding. The seventh terminal case (Case 14) is not included in these studies because only one determination was performed prior to death. This serum gave a "flocculation" gamma globulin value of 1.76 gm. per 100 ml. and a T.S./G.G. ratio of 1.39. Hence, this case would also fit into the terminal category on the basis of these findings although clinically it was classified as moderately advanced active disease. Such findings indicate that there is a decrease in the beta globulins (or lipoproteins) just prior to death since no increase in the gamma globulin value was obtained in these cases. Such a decrease in the globulin fraction one or two weeks before death has previously been reported by Sato (59). As seen in table 4 (*b*), stabilized cases of far advanced tuberculosis showed little change in either the "flocculation" gamma globulin values or in the T.S./G.G. ratio when studied periodically.

As seen from the data in table 4 (*d*), patients with far advanced disease becoming progressively worse yield findings which approximate the terminal stage. Conversely, patients in whom the disease is improving (table 4 (*c*)) give protein flocculation results which change in the direction of the group with minimal tuberculosis. From the electrophoretic data for the minimal group, it can be observed that both the beta globulins and the alpha-2 globulins are significantly increased above normal as evidenced by a significance ratio above 7. In other words, the odds against obtaining a mean of similar groups which is outside the probable error are 445,000 to 1. The increase in the beta globulin component is believed by some investigators (60) to be associated with the process of tissue healing which is probably continuing to take place in these arrested cases. The present writers have found but little difference in the mean alpha-2 globulin values between the moderately advanced group and the minimal arrested group. Consequently, measurement in the change of this component either directly by electrophoresis or indirectly by the perchloric acid-tryptophan polysaccharide method of Seibert, Pfaff, and Seibert (61) appears to be of questionable validity in differentiating between these two groups. The data from the present study confirm the findings of Seibert and her associates (24) and of Gerstl and co-workers (43) that gamma globulin values constitute the best over-all indicator of the clinical condition of a patient with pulmonary tuberculosis.

In conclusion, it is believed that the quantitative photometric microprocedures for gamma globulin developed in the present laboratory lend themselves as useful tools for evaluation and prognosis of pulmonary tuberculosis. Moreover, the T.S./G.G. ratio furnishes additional information concerning the clinical status of such patients.

Since many of the patients discussed in this paper are now being treated with various isonicotinic acid derivatives (63 patients), the study is being extended in order to evaluate objectively the effect of these newly reported drugs in the treatment of pulmonary tuberculosis.

SUMMARY

Previously published quantitative, photometric microprocedures for serum gamma globulin have been applied to the study of protein changes in approximately 100 cases of pulmonary tuberculosis with varying degrees of severity.

Periodic studies were also performed in 15 selected cases during a one-year period in order to compare the laboratory findings with the clinical course of the disease.

These quantitative protein flocculation methods were compared in approximately 50 cases with chemical salt fractionation and electrophoretic procedures.

All patients with active pulmonary tuberculosis, including those with minimal lesions, showed elevation of the gamma globulin. It is believed that this increase has diagnostic significance in conjunction with other procedures in the detection of early pulmonary tuberculosis and in evaluating arrested cases.

Gamma globulins increase (and the chemical and electrophoretic A/G ratios decrease) significantly with the severity of the disease and tend to return to normal limits when the tuberculosis becomes arrested. These determinations are therefore useful for the prognosis of patients with pulmonary tuberculosis. The alpha-2 and beta globulins, which remain elevated even in the arrested cases, are not suitable prognostic indicators.

A new entity, the total serum/gamma globulin ratio, is described which reflects primarily changes in the beta globulin fraction. In patients studied serially this ratio decreased markedly with the increasing severity of the disease. Six of 7 terminal cases showed markedly elevated gamma globulin and significantly decreased ratio values. Conversely, in patients who showed improvement, decreasing gamma globulin and increasing total serum/gamma globulin ratio values were noted when followed serially.

SUMARIO

Las Fracciones Electroforética y Química de la Seroalbúmina en la Tuberculosis Pulmonar

Los microprocedimientos fotométricos, cuantitativos, previamente publicados para la globulina gamma del suero sanguíneo, han sido aplicados al estudio de las alteraciones de la proteína en unos 100 casos de tuberculosis pulmonar más o menos grave. También se ejecutaron durante un año estudios seriados en 15 casos seleccionados, a fin de comparar los hallazgos de laboratorio con la evolución clínica de la enfermedad.

Esas técnicas cuantitativas para medir la floculación de la proteína fueron comparadas en unos 50 casos con los procedimientos de la electroforesis y de la fraccionación de las sales químicas.

Todos los enfermos que tienen tuberculosis pulmonar activa, incluso lesiones mínimas, muestran elevación de la globulina gamma. Parece que ese aumento posee importancia diagnóstica, en unión de otros procedimientos, en el descubrimiento de la tuberculosis pulmonar incipiente y la justipreciación de casos estacionados.

Las globulinas gamma aumentan (y las proporciones química y electroforética de A/G disminuyen) significativamente con la gravedad de la enfermedad y tienden a volver a límites normales al estacionarse el caso. Esas determinaciones son, por lo tanto, útiles para el pronóstico en los tuberculosos pulmonares. Las globulinas alfa-2 y beta, que permanecen elevadas hasta en los casos estacionados, no son indicadores adecuados para el pronóstico.

Descríbese una nueva entidad, la proporción de suero total/globulina gamma en el coágulo, que refleja primordialmente las alteraciones en la fracción de globulina beta. En los enfermos estudiados en serie, esa proporción disminuyó agudamente a medida que se agravaba la dolencia. Seis de 7 casos terminales revelaron notable elevación de la globulina gamma y disminución significativa de las cifras de la proporción. En cambio, en los enfermos que mostraron mejoría, notáronse, al observarlos continuamente, valores decrecientes de la globulina gamma y ascendentes en la proporción de suero total/globulina gamma en el coágulo.

RESUME

Fractions électrophorétiques et chimiques de la protéine sérique dans la tuberculose pulmonaire

Les micro-méthodes quantitatives photométriques pour la globuline gamma sérique, qui ont été précédemment publiées, ont été appliquées à l'étude d'environ 100 cas de tuberculose de différents degrés de sévérité. Pendant une période d'une année, il a également été procédé à des études en série sur 15 cas sélectionnés, afin de comparer les résultats fournis par le laboratoire et le cours clinique de la maladie.

Ces méthodes quantitatives de floculation protéique ont été comparées dans approximativement 50 cas avec le fractionnement chimique des sels et les procédés d'électrophorèse.

Tous les malades présentant une tuberculose pulmonaire en activité, y compris ceux présentant des lésions discrètes, montrent une augmentation de la globuline gamma. Les auteurs sont d'avis que cet accroissement a une signification diagnostique lorsqu'il s'ajoute aux autres épreuves dans le dépistage de la tuberculose pulmonaire au début, et aussi dans l'évaluation des cas stabilisés.

Les globulines gamma augmentent (et les rapports chimiques et électrophorétiques A/G s'abaissent) de façon significative avec la sévérité de l'affection; elles tendent à reprendre leur valeur normale quand le cas se stabilise. Ces déterminations sont donc utiles pour établir le pronostic chez les sujets atteints de tuberculose pulmonaire. Les globulines alpha-2 et bêta qui demeurent élevées, même dans les cas stabilisés, ne constituent pas des indicateurs adéquats pour le pronostic.

Une nouvelle entité, le rapport sérum totale/globuline gamma (total serum/gamma globulin ratio), est décrite; ce rapport réflète surtout les modifications de la fraction globuline bêta. Chez les malades étudiés en série, ce rapport s'abaisse nettement lorsque la sévérité de l'affection augmente. Sur les 7 cas mortels, 6 montraient une élévation marquée de la teneur en globuline gamma et une diminution nette de la valeur du rapport. Au contraire, chez les sujets qui témoignaient d'une amélioration, les études en série montraient une diminution de la globuline gamma et une élévation du rapport sérum totale/globuline gamma du caillot.

Acknowledgment

The authors wish to acknowledge the assistance of Mrs. Iowa Marable, Chief Technician of the U. S. Public Health Service Hospital, for her cooperation in drawing the many blood samples used in these studies, and to express their gratitude to Miss Renee Eisner for the typing of the manuscript. We are also grateful to Mr. Herbert Fischler for the photographic illustrations.

REFERENCES

(1) Dubos, R. J., and Davis, B. D.: Factors affecting the growth of tubercle bacilli in liquid media, J. Exper. Med., 1946, *83*, 409.

(2) Goldie, H.: Use of Dubos medium for culture of *M. tuberculosis* from sputum, Proc. Soc. Exper. Biol. & Med., 1947, *65*, 210.

(3) Middlebrook, G., and Dubos, R. J.: Specific serum agglutination of erythrocytes sensitized with extracts of the tubercle bacillus, J. Exper. Med., 1948, *88*, 521.

(4) Fleming, J. W., Runyon, E. H., and Cummings, M. M.: An evaluation of the hemagglutination test for tuberculosis, Am. J. Med., 1951, *10*, 704.

(5) Kirby, W. M. M., and Burnell, J. M.: Evaluation of a hemagglutination test for the diagnosis of active tuberculosis (abstract), Am. J. Med., 1951, *10*, 761.

(6) Hentel, W., and Guilbert, G. D.: Results in the hemagglutination test for tuberculosis, J. Lab. & Clin. Med., 1952, *39*, 426.

(7) Eichelberger, L., and McCluskey, K. L.: Chemical studies in tuberculosis; plasma proteins, cholesterol and corpuscle volume, Arch. Int. Med., 1927, *40*, 831.

(8) Luthy, F.: Klinische Untersuchungen über Serumeiweisskörper bei Lungentuberkulose, Schweiz. med. Wchnschr., 1927, *57*, 993.

(9) Dufort, A., and Moreau, R.: Albumines et quotient albumieux du serum chez la tuberculose pulmonaire, Presse méd., 1930, *38*, 843.

(10) Olivier, H. R., and Sliosberg, A.: Etude des proteines du serum au cours de la tuberculose pulmonaire, Rev. de la tuberc., 1931, *12*, 5.

(11) Manicatide, M., Bratescom A., and Rozenkranz, B.: Les modifications de l'equilibre proteique du serum dans les tuberculoses infantiles, Compt. rend. Soc. de biol., 1930, *105*, 45.

(12) Masson, P.: Les albumines du serum dans la tuberculose pulmonaire, Rev. de la tuberc., 1931, *12*, 828.

(13) Spehl, P., and Thys, F.: Cociente albuminas-globulinas en los tuberculosos; comparacion con la sedimentacion globular, Rev. de hig. y de tuberc., 1934, *27*, 35.

(14) Gutman, A. B., Gutman, E. B., Jillson, R., and Williams, R. D.: Acid-base equivalence of the blood in diseases associated with hyperglobulinemia; with special reference to lymphogranuloma inguinale and multiple myeloma, J. Clin. Investigation, 1936, *15*, 475.

(15) Lenci, E.: La proteinemia nella tuberculosi polmonare, Riv. di pat. e clin. d. tuberc., 1937, *15*, 322.

(16) Vignati, J., and Rauchenberg, M.: Bence-Jonessche Eiweisskörper im Blute, Klin. Wchnschr., 1937, *16*, 62.

(17) de Vries, A.: Thesis, Amsterdam, 1938.

(18) Howe, P. E.: The use of sodium sulfate as the globulin precipitant in the determination of proteins in blood, J. Biol. Chem., 1921, *49*, 93.

(19) Tiselius, A.: Electrophoresis of serum globulin: II. Electrophoretic analysis of normal and immune sera, Biochem. J., 1937, *31*, 1464.

(20) Tiselius, A., and Kabat, E. A.: An electrophoretic study of immune sera and purified antibody preparations, J. Exper. Med., 1939, *69*, 119.

(21) Svensson, H.: Fractionation of serum with ammonium sulfate and water dialysis studied by electrophoresis, J. Biol. Chem., 1941, *139*, 805.

(22) Gutman, A. B., Moore, D. H., Gutman, E. B., McClellan, V., and Kabat, E. A.: Fractionation of serum proteins in hyperproteinemia with special reference to multiple myeloma, J. Clin. Investigation, 1941, *20*, 765.

(23) Petermann, M. L., Young, N. F., and Hogness, K. R.: A comparison of the Howe and the electrophoretic methods for the determination of plasma albumin. J. Biol. Chem., 1947, *169*, 379.

(24) Seibert, F. B., Seibert, M. V., Atno, A. J., and Campbell, H. W.: Variation in protein and polysaccharide content of sera in the chronic diseases, tuberculosis, sarcoidosis and carcinoma. J. Clin. Investigation, 1947, *26*, 90.

(25) Lenci, E.: Rapport tra quadro proteico e reazioni di labilita colloidale del siero (reazione di Matety, Darany, Baum a Weltmann) nella tuberculosi pulmonare, Gazz. d. osp., 1937, 58, 608.

(26) Levinson, S. A., and Klein, R. I.: The Weltmann serum coagulation reaction, Am. Rev. Tuberc., 1938, *37*, 200.

(27) de Vries, A.: On the connection between the Takata-Jezler reaction and its variant, the Mancke-Sommer reaction and the globulin fractions of the blood, Acta med. Scandinav., 1938-1939, *98*, 95.

(28) de Vries, A.: On the connection between the formol gel reaction and the blood proteins, Acta med. Scandinav., 1939, *99*, 425.

(29) Bing, J.: Further investigations on hyperglobulinemia, Acta med. Scandinav., 1940, *103*, 547.

(30) Befani, A.: Su una nuova reazione di flocculazione del plasma per la diagnosi e per la valutazione delle forme tubercolari ricerche di controllo, Lotta contro la tuberc., 1950, *20*, 321.

(31) Pedrazini, A., Salvidio, E., and Wuhrmann, F.: Il significato di alcune reazioni protidemiche nella tuberculosi pulmonare, Minerva med., 1950, *41*, 81.

(32) Huerga, J. de la, Popper, H., Franklin, M., and Routh, J. I.: Comparison of the results of gamma globulin and zinc sulfate turbidity test with electrophoretic determination of the gamma globulins, J. Lab. & Clin. Med., 1950, *35*, 466.

(33) Schmid, R.: The zinc turbidity test and its clinical application, J. Lab. & Clin. Med., 1950, *36*, 52.

(34) Wuhrmann, F. H., and Wunderly, C.: The cadmium reaction, J. Lab. & Clin. Med., 1949, *34*, 1162.

(35) Saifer, A.: Protein flocculation reactions: A physico-chemical approach, Am. J. Med., 1952, *13*, 730.

(36) Jager, B. V., and Nickerson, M.: Simple quantitative chemical method for estimating gamma globulin in human serum, J. Biol. Chem., 1948, *173*, 683.

(37) Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F.: Studies in serum proteins; rapid procedure for estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin, in 1.0 ml of serum, Am. J. Clin. Path., 1948, *18*, 723.

(38) Kibrick, A. C., and Blonstein, M.: Fractionation of serum into albumin and alpha, beta and gamma globulin by sodium sulphate, J. Biol. Chem., 1948, *176*, 983.

(39) Svensson, H.: Electrophoresis by the moving boundary method: A theoretical and experimental study, Ark. Kemi, Mineral. o. Geol., 1946, *22A*, No. 10.

(40) Majoor, C. L. H.: The possibility of detecting individual proteins in blood serum by differentiation of solubility curves in concentrated sodium sulfate solutions: II. Comparison of solubility curves with results of electrophoresis experiments, J. Biol. Chem., 1947, *169*, 583.

(41) Dole, V. P.: Electrophoretic patterns of normal plasma, J. Clin. Investigation 1944, *23*, 708.

(42) Huerga, J. de la, and Popper, H.: Estimation of serum gamma globulin concentration by turbidimetry, J. Lab. & Clin. Med., 1950, *35*, 459.

(43) Gerstl, B., Kidder, L. E., Jacobs, L. G., and Ramornio, P. M.: Serum gamma globulin estimations in patients with pulmonary disease, Nat. Tuberc. A. Tr., 1950, p. 128.

(44) Seibert, F. B.: Discussion of paper by Gerstl *et al.* (43), Nat. Tuberc. A. Tr., 1950, p. 151.

(45) Saifer, A., and Zymaris, M.: Photometric microdetermination of human gamma globulin: I. Development of a quantitative flocculation-ninhydrin procedure, J. Clin. Investigation, 1952, *31*, 1.

(46) Saifer, A., Zymaris, M., and Berger, H.: Photometric microdetermination of human gamma globulin: II. Comparison of quantitative flocculation-ninhydrin method with electrophoretic method, J. Clin. Investigation, 1952, *31*, 22.

(47) National Tuberculosis Association: Diagnostic Standards and Classification of Tuberculosis, 1940 edition, New York, N. Y.

(48) Hughes, F. J., Mardis, R. E., Dye, W. E., and Tempel, C. W.: Combined intermittent regimens in the treatment of non-miliary pulmonary tuberculosis, Dis. of Chest, 1952, *21*, 1.

(49) Philpot, J. S. L.: Direct photography of ultracentrifuge sedimentation curves, Nature, 1938, *141*, 283.

(50) Svensson, H.: Direkte photographische Aufnahme von Elektrophorese-diagrammen, Kolloid-Z., 1939, *87*, 181; Theorie der Beobachtungsmethode der gekreuzten Spalte, Kolloid-Z., 1940, *90*, 141.

(51) Longsworth, L. G.: Recent advances in the study of proteins by electrophoresis, Chem. Rev., 1942, *30*, 323.

(52) Campbell, W. R., and Hanna, M. I.: The albumins, globulins and fibrinogen of serum and plasma, J. Biol. Chem., 1937, *119*, 15.

(53) Kingsley, G. R.: Direct biuret method for determination of serum proteins as applied to photoelectric and visual colorimetry, J. Lab. & Clin. Med., 1942, *27*, 840.

(54) Gornall, A. G., Bardawill, C. J., and David, M. M.: Determination of serum proteins by means of the biuret reaction, J. Biol. Chem., 1949, *177*, 751.
(55) Reiner, M., Fenichel, R. L., and Stern, K. G.: Electrophoretic studies on the protein distribution in normal human serum, Acta haematol., 1950, *3*, 202.
(56) Maclagan, N. F.: Flocculation tests: Chemical and clinical significance, Brit. M. J., 1948, *2*, 892.
(57) Gavett, G. L.: A First Course in Statistical Method, McGraw-Hill, New York, 1937.
(58) Milne, J.: Serum protein fractionation; comparison of sodium sulfate precipitation and electrophoresis, J. Biol. Chem., 1947, *169*, 595.
(59) Sato, I.: Serum proteins in tuberculosis, Tohoku J. Exper. Med., 1933-1934, *22*, 111.
(60) Franklin, M., Popper, H., Steigman, F., and Kozoll, D. D.: Relation between structural and functional alterations of the liver, J. Lab. & Clin. Med., 1948, *33*, 435.
(61) Seibert, F. B., Pfaff, M. L., and Seibert, M. V.: A serum polysaccharide in tuberculosis and carcinoma, Arch. Biochem., 1948, *18*, 279.

THE BACTERICIDAL ACTION OF ISONIAZID, STREPTOMYCIN, AND TERRAMYCIN ON EXTRACELLULAR AND INTRACELLULAR TUBERCLE BACILLI[1]

G. B. MACKANESS[2] AND N. SMITH

(Received for publication September 24, 1952)

INTRODUCTION

None of the antituberculous drugs at present available will eliminate all tubercle bacilli from the organs of animals infected experimentally. Organisms that are both viable and virulent have been recovered from the tissues after prolonged treatment with streptomycin (1–6) and, probably, also with isoniazid (7, 8).

Three main possibilities present themselves to account for this failure of treatment. First, the bacilli may have developed resistance to the drug; second, they may be so situated that the drug does not reach them; or third, some of the bacilli may be in a metabolic state in which they are not susceptible.

Concerning the first of these possibilities, it is true that resistant strains are frequently recovered from patients treated with streptomycin, and they have also been found in patients treated with isoniazid (9). Moreover, both of these drugs readily yield resistant strains when *M. tuberculosis* is exposed to them *in vitro*. In experimental tuberculosis, however, strains of bacilli recovered from treated animals very seldom show increased resistance to the drug when tested *in vitro* (3, 5, 6, 8, 10); and, of the strains recovered by Beck and Yegian (11) from resected pulmonary tissue in man, all but one of those tested were found to be drug-susceptible. It is difficult, in the light of these findings, to give much weight to drug resistance as a primary factor in the failure to eliminate bacilli.

It is uncertain to what extent bacilli situated in necrotic areas are accessible to drugs. Bacilli contained within macrophages are certainly protected to a large extent from streptomycin, which penetrates poorly into cells (12) and is relatively ineffective against intracellular tubercle bacilli *in vitro* (13, 14). Isoniazid penetrates cells more freely (13, 15), and it will be shown below that it readily kills intracellular bacilli. Nevertheless, some bacilli seem to escape the action of isoniazid in the animal body (7, 8).

Concerning the third of the alternatives listed above, there is very little evidence. *In vitro*, streptomycin has no significant action on tubercle bacilli in a medium in which the bacilli do not divide (16, 17), and it is possible that caseous areas provide comparable conditions. Tubercle bacilli in closed lesions in man may die out in the absence of any treatment by drugs (11, 18), suggesting that conditions there are not suitable for growth. Bacilli which persist for a time under these conditions may be unaffected by an otherwise bactericidal drug.

The last-named alternative is probably the one that is of most importance in considering the persistence of drug-sensitive bacilli in the tissues of animals dur-

[1] From the Sir William Dunn School of Pathology, Oxford, England.
[2] Research Fellow of the Australian National University, Canberra.

ing treatment. In general, lesions under effective chemotherapy are regressive and show signs of healing so that it is unlikely that the organisms in them are dividing. The resting state of such bacterial cells is particularly evident when viable bacilli are found in organs which have completely healed under treatment (1), showing that resting bacilli can exist *in vivo* in tissues other than those which are necrotic. When the host's defense system is ineffective even against non-dividing bacilli, no drug but one which is bactericidal and active against resting bacterial cells will rid the tissues of the parasite.

In the present work, the bactericidal activities of streptomycin and isoniazid have been studied. Since neither of these drugs alone is completely effective, their respective bactericidal activities have been compared with the bactericidal activity of the two drugs acting together at various concentrations. The effect upon both extracellular and intracellular tubercle bacilli has been examined. Several workers have used streptomycin and isoniazid together, but under conditions that can show only a bacteriostatic action (20, 21) or an action upon residual bacterial cells resistant to one or the other of the drugs (22). A third antituberculous drug, terramycin, was tried in view of its free passage into cells (14) but, as it proved to antagonize the action of isoniazid, it did not reach the stage of trial in macrophage preparations.

The present study falls into two parts: (*1*) survival of tubercle bacilli in Dubos-Davis medium when exposed to isoniazid with streptomycin or terramycin, or to each drug along; (*2*) survival of tubercle bacilli in living macrophages when the cells are exposed to streptomycin and isoniazid together or alone.

Materials and Method

The H37Rv strain of *M. tuberculosis* was used throughout. The inocula were prepared from shallow cultures grown in Dubos-Davis medium (23), containing 0.05 per cent Tween 80[3] and 0.25 per cent heat-inactivated bovine albumin fraction V (Armour). The cultures were shaken daily to promote dispersion and were harvested after eleven days. The growth was pooled and washed in fresh medium. The final suspension was lightly centrifuged to deposit the larger aggregates, and the density of the supernatant was adjusted according to particular requirements.

Cultures in Dubos-Davis liquid medium

To examine the survival of bacilli exposed to streptomycin and isoniazid singly, two series of decimal dilutions of the drugs were prepared in Tween-albumin medium; one containing streptomycin in concentrations ranging from 100 to 0.1 γ per ml. (final concentrations), the other containing isoniazid in concentrations ranging from 10 to 0.01 γ per ml. (final concentrations). The effect of combining the drugs was observed in two similar series, in which a final constant concentration of 0.1 γ of isoniazid per ml. was added to the streptomycin series and a final constant concentration of 1.0 γ of streptomycin per ml. was added to the isoniazid series. The respective bacteriostatic activities of these final constant concentrations of the two drugs were approximately equal. The controls included cultures without added drugs and cultures containing only the final constant concentrations used in the two combination series. In order to allow for the risk of contamination during the

[3] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

frequent sampling operations, a duplicate set of cultures was prepared. Actually, this duplicate set remained undisturbed throughout the course of the experiment.

The technique used for viable counts was that described by Fenner, Martin, and Pierce (24) with only slight modification, and later shown by Fenner (25) to yield reliable estimates of the number of viable units of the H37Rv strain, and other strains, of *M. tuberculosis*. After thorough mixing, a 0.5 ml. sample was taken from each culture and diluted with 4.5 ml. of distilled water containing 0.1 per cent bovine albumin. A further serial 1 in 10 dilution was carried out in the same diluent. The samples were taken at the commencement of the experiment, after twelve hours of incubation at 37° C., and after one, two, four, eight, eighteen, and thirty-two days of incubation.

A range of the dilutions and the undiluted culture were inoculated on the surface of oleic-acid–albumin-agar plates (24). Ten replicate drops of 0.02 ml. were allowed to settle onto the surface of each plate; this was then sealed with adhesive cellulose tape and paraffin wax. Colony counts were made with a plate microscope (× 35) using incident illumination. These were carried out when growth appeared and at intervals until a maximum count had been obtained. After thirty-two days, all cultures which were thought to contain no viable bacilli were inoculated into normal guinea pigs in order to prove sterility.

MACROPHAGE CULTURES

Principle: The bactericidal action of streptomycin and isoniazid upon intracellular tubercle bacilli was determined by exposing infected macrophages to a nutrient medium containing these drugs and subsequently transferring the entire macrophage population to oleic-acid–albumin-agar. With phase-difference microscopy, an estimate was then made of the number of macrophage residues which had yielded colonies of tubercle bacilli on further incubation at 37°C. (figures 1–4).

Procedure: The technique of maintaining an infected population of mononuclear phagocytes in special culture chambers and of transferring them to oleic acid–albumin-agar has already been described (14). In these experiments, the use of Hanks' solution (26), containing 15 per cent lipoid-free rabbit serum, avoided the necessity for frequent changes of medium; the chambers were filled with a suspension containing only 200 cells per cu. mm. as a sparser cell population than that previously used was required to allow room for the development of discrete colonies of tubercle bacilli after transfer of the cell residues to the solid medium.

Infected cultures were prepared from normal rabbit macrophages, more than 80 per cent of which were estimated to contain an average of 5 tubercle bacilli per cell. Two procedures were adopted. In one, the cultures were exposed to one concentration of streptomycin or isoniazid for varying periods of time. In the other, the cultures were exposed for a fixed period of time to varying concentrations of the drugs. Duplicate macrophage cultures were exposed for intervals of one, two, four, and eleven days to 10 γ of streptomycin per ml. of culture medium, to 1.0 γ of isoniazid, and to 10 γ of streptomycin plus 1.0 γ of isoniazid. Duplicate cultures were also exposed for a fixed period of four days to 100, 10, and 1.0 γ of streptomycin per ml.; to 10, 1.0, and 0.1 γ of isoniazid per ml.; and to concentrations of streptomycin plus isoniazid of 100/10, 10/1.0, and 1.0/0.1 γ per ml., respectively.

After the specified period of incubation, the culture medium was replaced three times in one hour by medium free from antibacterial substances. This effected a 1:1,000 dilution of the original drug concentration. After the last medium change, the cultures were left for twenty-four hours at 37°C. All medium was then withdrawn, and the macrophages were left to dry in contact with the coverslip. When dry, they were covered with a very thin layer of oleic-acid–albumin-agar at 50°C. The chambers were then sealed and incubated at 37°C. until conspicuous colonies had developed or until it was certain that none would develop.

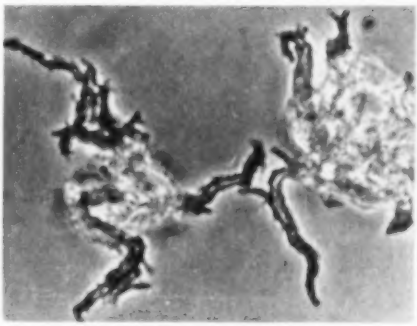
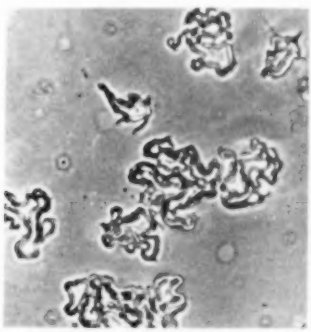


FIG. 1. (Left) From a macrophage culture which, for eleven days, had contained 1.0 γ of isoniazid per ml., photographed immediately after transfer to oleic acid–albumin agar. The photomicrograph shows a binucleate macrophage residue containing ill-defined tubercle bacilli which had remained inhibited and which failed to yield colonies on further incubation. (Phase-contrast, $\times$ 1,000)

FIG. 2. (Right) From a macrophage culture which, for four days, had contained 10 γ of streptomycin plus 1.0 γ of isoniazid per ml., photographed ten days after transfer to oleic-acid–albumin-agar. The preparation shows the appearance of colonies of tubercle bacilli growing from two macrophage residues; the body of the macrophage has been replaced by a coiled mass of bacilli which projects from its surface in small cords. (Phase-contrast, $\times$ 300)



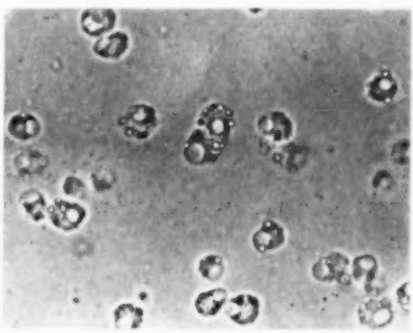


FIG. 3. (Left) From a control macrophage culture which had contained no drug and which had been transferred to oleic-acid–albumin-agar at the beginning of the experiment. The preparation shows the appearance of well-developed colonies of tubercle bacilli which have obliterated the macrophage residues. (Phase-contrast, $\times$ 100)

FIG. 4. (Right) From a macrophage culture which, for eleven days, had contained 1.0 γ of isoniazid per ml., photographed fourteen days after transfer to oleic-acid–albumin-agar. The photomicrograph shows the appearance of macrophage residues which failed to yield colonies on further incubation and is from the same culture as figure 1. (Phase-contrast, $\times$ 100)

Under normal conditions, colonies can be detected after four days' incubation. In this instance all cultures were incubated for at least ten days, and some for much longer.

In order to count the colonies, the chambers were filled with formalin in saline to make the chamber more or less optically homogeneous. With phase difference microscopy, the

culture was then scanned and each macrophage residue was examined for evidence of bacterial growth. The percentage of residues yielding colonies was estimated from a random sampling of between 300 and 1,000. As two or more cultures were prepared from each drug concentration, these percentages were averaged.

An estimate of the number of macrophages infected initially was made from several cultures, both with and without drug, which were transplanted to solid medium at the commencement of the experiment.

TABLE 1

INHIBITION OF THE GROWTH OF M. TUBERCULOSIS (STRAIN H37Rv GROWN IN TWEEN-ALBUMIN MEDIUM) BY STREPTOMYCIN AND ISONIAZID, SINGLY AND IN COMBINATION*

DRUG CONCENTRATION		DAYS OF INCUBATION				
Streptomycin	Isoniazid	6	8	18	25	32
γ per ml.	γ per ml.					
100.0	—	− −	− −	− −	− −	− −
10.0	—	− −	− −	− −	− −	− −
1.0	—	− −	− −	− −	+ +	+ +
0.1	—	+ +	+ +	+ +	+ +	+ +
—	10.0	− −	− −	− −	− −	− +
—	1.0	− −	− −	− −	+ +	+ +
—	0.1	− −	− −	+ +	+ +	+ +
—	0.01	− −	+ +	+ +	+ +	+ +
100.0	0.1	− −	− −	− −	− −	− −
10.0	0.1	− −	− −	− −	− −	− −
1.0	0.1	− −	− −	− −	− −	− −
0.1	0.1	− −	− −	+ +	+ +	+ +
—	0.1	− −	− −	+ +	+ +	+ +
1.0	10.0	− −	− −	− −	− −	− −
1.0	1.0	− −	− −	− −	− −	− −
1.0	0.1	− −	− −	− −	− −	− −
1.0	0.01	− −	− −	− −	− −	+ +
1.0	—	− −	− −	− −	+ +	+ +
Control		+ +	+ +	+ +	+ +	+ +

* Sampled and unsampled cultures recorded in separate columns

RESULTS

CULTURES IN DUBOS-DAVIS MEDIUM

Streptomycin and Isoniazid

Inhibition of growth: The titers for simple growth inhibition in the series of cultures of *M. tuberculosis* from which samples were taken, and in the duplicate series which remained undisturbed, are recorded in table 1. Growth appeared in increasingly higher concentrations of isoniazid as the period of incubation was extended. Only one of the two cultures containing 10 γ of isoniazid per ml. became sterile; the other, an unsampled culture, showed growth after thirty-two days of incubation.

A concentration of streptomycin of 1.0 γ per ml., which is itself subinhibitory, was effective in preventing the growth of bacilli in all concentrations of isoniazid except 0.01 γ per ml., at which concentration growth was merely delayed. A similar slight potentiation of bacteriostatic effect by combination of the two drugs can be seen in the series in which a constant level of isoniazid was maintained.

TABLE 2

LOGARITHM OF THE NUMBER OF VIABLE UNITS PER ML. OF M. TUBERCULOSIS (STRAIN H37Rv), EXPOSED IN DUBOS-DAVIS MEDIUM TO VARYING CONCENTRATIONS OF STREPTOMYCIN AND ISONIAZID, SINGLY AND IN COMBINATION

DRUG CONCENTRATION		DAYS OF INCUBATION							
Streptomycin	Isoniazid	0	35	1	2	4	8	18	32
γ per ml.	*γ per ml.*								
100.0	—		5.18	4.81	3.63	3.18	0	0	0
10.0	—		5.36	5.02	4.32	3.72	2.18	0	0
1.0	—		6.19	6.06	5.35	4.05	3.36	4.25	+
0.1	—		6.19	6.17	6.23	6.65	+	+	+
—	10.0		6.09	6.06	6.08	4.28	3.00	1.00	0
—	1.0		6.10	6.15	5.56	4.24	3.08	4.20	+
—	0.1		6.21	6.18	5.96	4.21	3.40	6.30	+
—	0.01	6.20 ± 0.03	6.19	6.21	6.07	5.58	+	+	+
100.0	0.1		5.12	4.85	4.24	2.65	0	0	0
10.0	0.1		5.51	5.00	4.37	3.78	2.18	0	0
1.0	0.1		6.14	5.15	4.28	3.79	2.81	0	0
0.1	0.1		6.13	6.07	5.63	4.08	3.47	+	+
—	0.1		6.14	6.08	5.78	4.23	3.40	+	+
1.0	10.0		6.16	5.13	4.23	3.53	0	0	0
1.0	1.0		6.16	5.12	4.38	3.54	2.70	0	0
1.0	0.1		6.10	5.12	4.42	3.88	2.81	1.00	0
1.0	0.01		6.19	5.81	5.21	4.13	3.32	3.15	+
1.0	—		6.19	6.10	5.74	4.58	3.80	6.27	+
Control			6.21	6.22	6.29	6.57	+	+	+

\+ = visible growth
0 = sterility proved by animal inoculation

Similar observations have been made by Ilavsky (20). These findings would appear to indicate that each of the two drugs has an effect upon bacilli which are potentially resistant to the other.

Survival of bacilli: The survival rate of bacilli exposed to varying concentrations of streptomycin and isoniazid, singly and in combination, is recorded in table 2. The carry-over of drug at the plating-out operation does not appear to have interfered seriously with the viable counts. Comparison between counts at dilu-

tions of 10^{-1} and 10^{-2}, whenever these could conveniently be made, showed very close agreement.

The effect of varying concentrations of streptomycin upon the survival of tubercle bacilli in Dubos-Davis medium is shown in figure 5. It will be seen that the death rate varies directly as the drug concentration. This finding is in accord with that of Garrod (27). It must be borne in mind, however, that the results of different workers cannot be compared on a quantitative basis. Apart from variations in technique, the degree of dispersal of the bacilli in the original inoculum profoundly affects the revealed death rate, especially in the early stages. As has been pointed out by Eagle, Fleischman, and Levy (28), the true death rate can

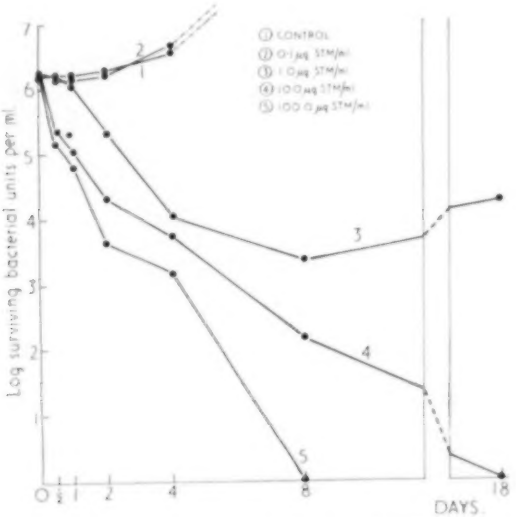


FIG. 5. Survival curves of bacterial units of *M. tuberculosis* (H37Rv) during exposure at 37° C. in Dubos-Davis medium to varying concentrations of streptomycin.

be calculated only if the distribution of the bacilli within the bacterial units is known. In these experiments, although the bacilli were well dispersed, the inoculum was far from unicellular. Aggregates of 2 and 3 bacilli predominated, but many units of larger size were also present. The distribution of the bacilli in the Dubos-Davis cultures was therefore similar to that in the macrophage cultures, where there is a grouping of the bacilli within the cells.

Above 0.1 γ per ml., the bactericidal effect of isoniazid appears to be independent of the drug concentration (figure 6). It seems, however, that resistant bacilli are as likely to appear at higher concentrations as at lower ones. This point has been emphasized by Middlebrook (22), although the studies of Szybalski and Bryson (29) with *M. ranae* show some grading of response with different concentrations of the drug. Their data, however, relate only to extreme degrees of resistance.

The survival curves for isoniazid (figure 6) show a delay of forty-eight hours before the onset of any marked fall in the viable count. The presence of bacterial aggregates renders this part of the curve insensitive to any but large changes in the bacterial population. The difference in the effect produced by isoniazid, compared with that of streptomycin, probably reflects a difference in the mode of action of the two drugs. It seems likely that streptomycin in high concentrations exerts a direct lethal action upon the resting tubercle bacillus. Low concentrations of streptomycin, on the other hand, do not appear to differ quantitatively from isoniazid in their bactericidal effect (figure 7).

The survival curve of bacilli exposed simultaneously to both streptomycin and isoniazid shows a consistent deflection at all concentrations at which the action

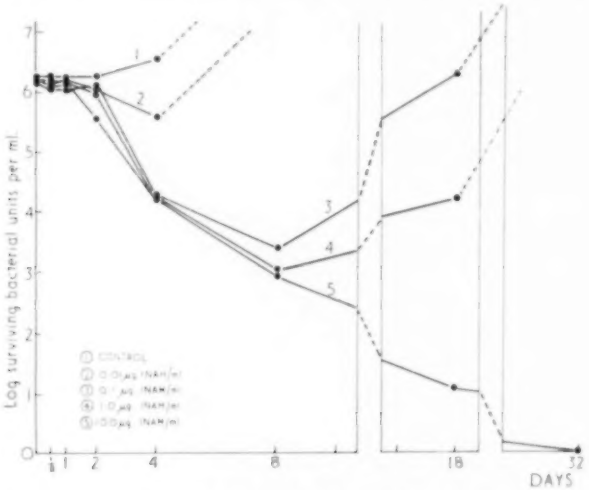


FIG. 6. Survival curves of bacterial units of *M. tuberculosis* (H37Rv) during exposure at 37° C. in Dubos-Davis medium to varying concentrations of isoniazid.

of one drug does not overwhelmingly preponderate. The effect of combining the two drugs at concentrations of 1.0 γ of streptomycin and 0.1 γ of isoniazid per ml. of culture medium is shown in figure 7. It will be seen that there is a pronounced fall in the viable count soon after the commencement of incubation. Within the first twenty-four hours, the two drugs in combination killed more than 90 per cent of the viable units present in the inoculum. This immediate acceleration in bactericidal effect is probably of therapeutic significance, for it is equal to the effect produced by at least ten times the concentration of either of the two drugs acting singly. It is perhaps even more important that the survival curve for the combination of the two drugs continues to fall to sterility, whereas either drug alone permits the outgrowth of resistant variants as early as the eighteenth day. As little as 0.01 γ of isoniazid per ml., when combined with 1.0 γ of streptomycin per ml., produced the same deflection in the survival curve al-

though in this instance the curve did not continue to fall to sterility. At none of the concentrations at which the two drugs were combined was any interference found between them. It can be seen, however, that, when the action of one drug predominates, no effect is produced upon the survival by addition of a small concentration of the other.

The combination of streptomycin and isoniazid in concentrations of 1.0 and 0.1 γ per ml., respectively, occurred twice, once in the series in which a constant level of streptomycin was maintained and again in the series in which a constant level of isoniazid was maintained. The fact that the survival curves for these

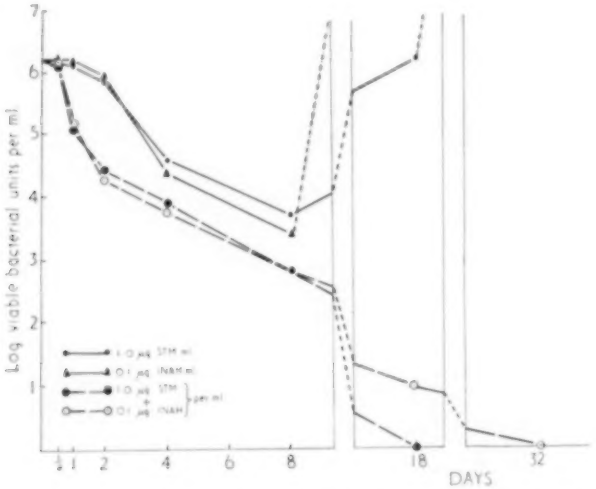


FIG. 7. Survival curves of bacterial units of *M. tuberculosis* (H37Rv) during exposure at 37° C. in Dubos-Davis medium to subinhibitory concentrations of streptomycin and isoniazid, singly and in combination. The combination of 1.0 γ of streptomycin plus 0.1 γ of isoniazid per ml. occurred in both the constant streptomycin and constant isoniazid series. The survival curves of both these cultures are included in the graph to indicate the reproducibility of results.

cultures were identical in both series indicates the reproducibility of these results (figure 7).

Terramycin and Isoniazid

The bactericidal actions of terramycin and isoniazid, both singly and in combination, were investigated under similar conditions except that a larger inoculum of very highly dispersed bacilli was used. It may be seen from figure 8 that a concentration of 10 γ of terramycin hydrochloride per ml. has a very limited bactericidal action. When terramycin and isoniazid were used in combination, the bactericidal action of isoniazid was almost completely inhibited. Terramycin and similar drugs have been shown to interfere with the action of both streptomycin (30) and penicillin (31) when acting upon certain strains of microorgan-

isms. The finding that terramycin inhibits the bactericidal action of isoniazid suggests that the bacilli whose multiplication is prevented by terramycin are insensitive to isoniazid and that the latter drug is probably only bactericidal for tubercle bacilli in an active metabolic state.

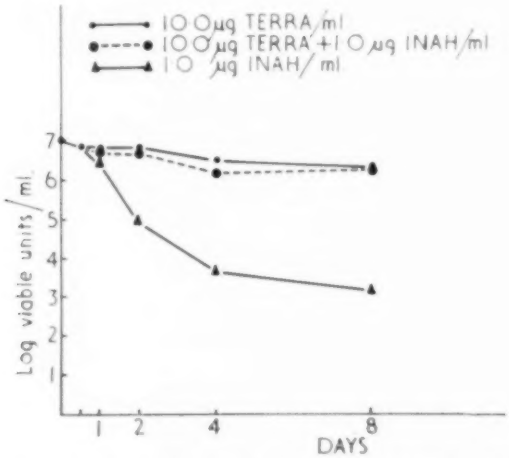


FIG. 8. Survival curves of bacterial units of *M. tuberculosis* during exposure at 37° C. in Dubos-Davis medium to subinhibitory concentrations of terramycin and isoniazid, singly and in combination. The bacteriostatic concentration of terramycin abolished most of the bactericidal action of isoniazid.

CULTURES OF BACILLI IN LIVING MACROPHAGES

To inhibit intracellular tubercle bacilli of the strain H37Rv, a streptomycin concentration of 25 γ per ml. must be maintained in the culture medium (14). It is likely that a biologically active concentration of streptomycin cannot be established inside macrophages with concentrations of less than 10 γ per ml. in the ambient medium. The last-named concentration approaches the maximum which can safely be reached for short periods in the blood of man. One microgram of isoniazid per milliliter, however, is easily achieved and sustained in body fluids. These were the concentrations, therefore, which were used to examine the effects of the two drugs upon the survival of intracellular tubercle bacilli.

In accordance with previous findings, cultures which contained 10 γ of streptomycin per ml., or less, died by the fifth day of incubation. All other cultures, with the exception of the controls containing no drug, remained in perfect condition. In those transferred to oleic-acid–albumin-agar on the eleventh day, there was no evidence of macrophage degeneration or of a reduction in the size of the macrophage population.

The percentage of macrophages which contained *viable* bacilli was determined in cultures which were transferred to solid medium at the commencement of incubation. In one culture which had contained no drug and in two which had con-

tained 10 γ of streptomycin and 1.0 γ of isoniazid per ml., before being washed free of these agents, 84 per cent of the macrophage residues produced colonies on the solid medium. The percentage of macrophages which had *taken up* bacilli was determined in a culture containing 100 γ of streptomycin and 10 γ of isoniazid per ml. of culture medium (figure 9). This culture was incubated for four days and then fixed and stained. Counts showed that bacilli were present in 87 per cent of the macrophages. This value agrees closely with that obtained for the percentage of macrophage residues containing viable bacilli, as determined by transfer to oleic-acid albumin-agar. The degree of redistribution which takes place in the macrophage cultures as a result of the death of some host cells and re-phagocytosis of liberated bacilli must, therefore, be very small. The close agreement between the two counts also shows that almost all of the bacilli in the original inoculum must have been viable. In the stained culture, there was a mean

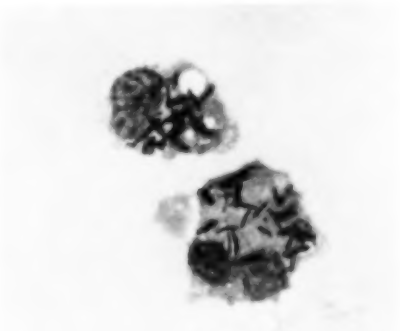


FIG. 9. Preparation of infected macrophage culture fixed after four days' incubation in the presence of 100 γ of streptomycin plus 10 γ of isoniazid per ml., showing the appearance of macrophage residues in the culture used to examine the distribution of intracellular tubercle bacilli. (Ziehl-Neelsen methylene blue, × 850)

of 7 bacilli per cell, the modal value being 2 bacilli per cell (range 0 to 30). Seventy-two per cent of the parasitized macrophages contained between 1 and 10 bacilli.

The effects of 10 γ of streptomycin per ml. of culture medium and of 1.0 γ of isoniazid, both singly and in combination, are shown in figure 10. Streptomycin alone produced an insignificant reduction in the number of macrophages infected. The interrupted line indicates death of the whole of the macrophage population after four days as a result of the outgrowth of the bacilli contained in the cells. Cultures treated with isoniazid or isoniazid plus streptomycin became sterile within eleven days. It was not unexpected that isoniazid alone produced this effect since the bacterial population did not exceed 60,000 and the chance of finding resistant bacilli in so limited a population is small (32). Furthermore, the action of normal macrophages on bacilli whose growth has been inhibited is as yet an unknown factor. If macrophages are infected with the avirulent strain H37Ra,

the entire bacterial population remains alive for several days. When transferred to solid medium after three days, almost all of the cells contain viable bacilli (figure 11). However, no viable bacilli can be found after six days of incubation

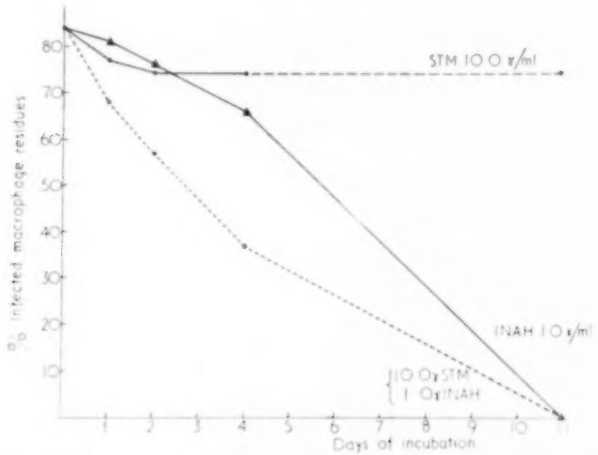


FIG. 10. Survival curves of intracellular tubercle bacilli during exposure of infected macrophage cultures to streptomycin and isoniazid, singly and in combination. The percentage of macrophages which contained viable bacilli are plotted for each drug concentration at intervals during incubation at 37° C.

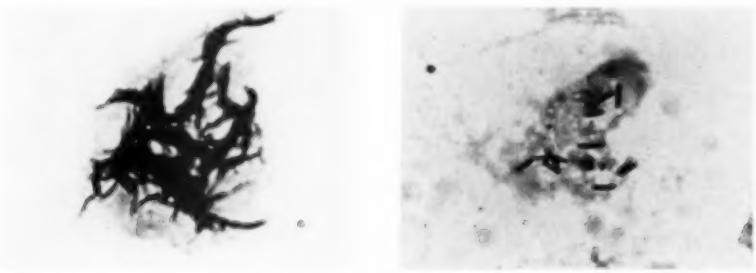


FIG. 11. (Left) Fixed preparation of macrophage culture infected with avirulent tubercle bacilli (H37Ra). The culture was transferred to solid medium after three days and, on further incubation, most of the macrophage residues produced colonies similar to the one shown.

FIG. 12. (Right) A macrophage residue, from the same culture series as that of figure 11, which contains avirulent bacilli which failed to grow on oleic acid albumin agar when transplanted after seven days' incubation in normal rabbit macrophages.

(figure 12), indicating that, in the absence of any immune system, the host cells are able to kill bacilli of this avirulent strain.

With the streptomycin concentration used (10 γ per ml.), the effective intracellular concentration was of the order of 0.5 γ per ml. In combination with

isoniazid, however, even this low concentration resulted in a significant acceleration in the death rate of the bacterial population. This is best seen on the fourth day (figure 10), when the number of surviving bacterial units in the presence of a combination of the drugs was approximately one-half the number of surviving units in the presence of either drug alone.

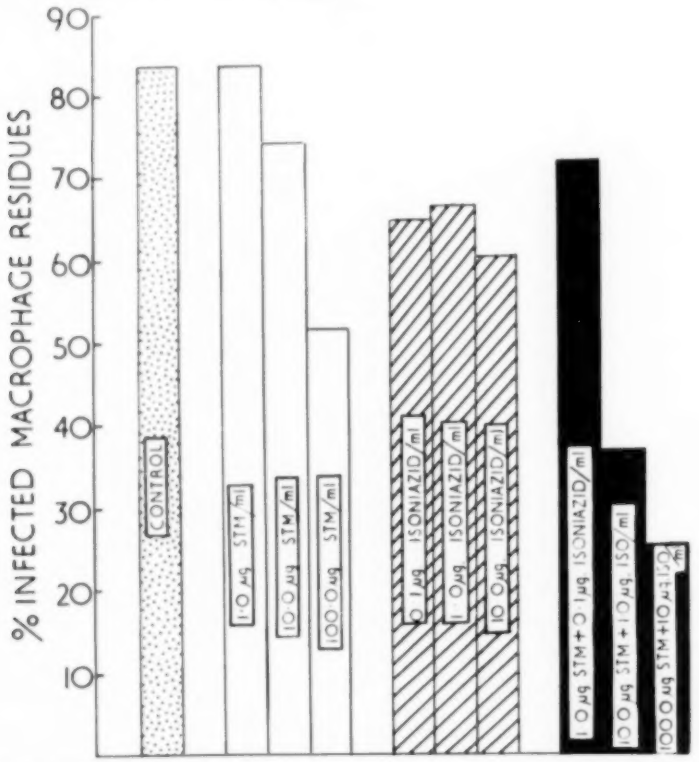


FIG. 13. Histogram showing the percentage of macrophages containing viable tubercle bacilli in cultures which had been exposed to varying concentrations of streptomycin and isoniazid, singly and in combination, for a fixed period of four days.

The effects of varying concentrations of streptomycin and isoniazid, singly and in combination, are shown in figure 13. In this instance all drugs were allowed to act for a fixed period of four days. As in the Dubos-Davis cultures, a graded response occurred to varying concentrations of streptomycin, but there was no constant relationship between the concentration of isoniazid and its bactericidal action. The combination of 1.0 γ of streptomycin and 0.1 γ of isoniazid per ml. resulted in no potentiation in drug action. At all other concentrations, however, the effect of combining the two drugs was similar to that obtained in Dubos-Davis medium.

Discussion

Three factors were considered in the introduction as important possible causes of bacilli persisting during the treatment of tuberculosis: (*1*) drug-resistant bacilli might be present; (*2*) the bacilli might be so situated that they are not reached by drugs; (*3*) the bacilli might be existing at such a low level of metabolic activity that they are not susceptible to drug action.

These three possible factors are now discussed in relation to the results of the present experiments.

To control the emergence of resistant bacilli, the use of two drugs has frequently been advocated. Demerec (33) and Hsie and Bryson (34) have said that the statistical chance that a bacterial variant will develop resistance to two drugs simultaneously is proportional to the product of the individual mutation rates to each drug singly, although this may not be true in general (35). The increased bacteriostatic action which was seen when streptomycin and isoniazid were used together in the present experiments shows that the growth of bacilli which were potentially resistant to one or other of the drugs was inhibited by simultaneous exposure to both. This evidence from the bacteriostatic end point was confirmed by studies of the survival of tubercle bacilli when exposed to streptomycin and isoniazid together in concentrations of each drug which were not completely bactericidal when used alone. Under these conditions, the viable count fell steadily and the cultures were eventually sterilized. This phenomenon probably indicates that the two drugs differ in their point of action on the bacterial cell. Jawetz and his co-workers (36) have been careful to point out that it is not a simple "sequential" effect in which one drug attacks those bacilli which remain unaffected by the other. Part of the evidence against such a view is the acceleration of the death rate which exposure to two synergistic drugs produces. This can only mean that the simultaneous attack has a more powerful bactericidal effect than the action of either drug alone; and it has important consequences which will be discussed later.

Many observations have shown that bacilli resistant to a particular drug can be found in any bacterial population, provided that that population is sufficiently large. On these grounds it has been suggested that, if one drug prevents multiplication in a bacterial population, the rate of emergence of bacilli resistant to a second drug will be much reduced simply by limiting the size of the exposed population. Such limitation (by a mechanism other than direct drug action) has in fact been shown to reduce the rate of emergence of drug-resistant strains (37). In simultaneous chemotherapy with two drugs, such a mechanism could be important only if some organisms were temporarily protected from the action of one or the other of the drugs. Intracellular tubercle bacilli in animals receiving streptomycin may be regarded as an example of such protected organisms. It may confidently be expected that isoniazid, because of its powerful action on intracellular bacilli, will largely eliminate this intracellular source of streptomycin-resistant variants. It is likely to prove more effective in this regard than para-aminosalicylic acid, which is almost completely inactive against intracellular tubercle bacilli (14).

Can it be expected that streptomycin, although penetrating cells poorly, will have some reciprocal action with isoniazid and help to eliminate intracellular bacilli which are potentially resistant to the latter? It was found that, when 10 γ of streptomycin per ml. of culture medium were present, enough penetrated the macrophages to potentiate the bactericidal effect of isoniazid so that probably it can also affect the rate of emergence of isoniazid-resistant strains from within the cell. However, a streptomycin concentration in the body fluids of less than 10 γ per ml. is not likely to be effective.

There is always great variation in the susceptibility of individual bacterial cells to a noxious agent when tested *in vitro* (38). The trend of survival curves (figures 5 and 6) during exposure to streptomycin and isoniazid shows this quite clearly. Hinshelwood (39) has pointed out that this differing susceptibility probably reflects differences in the metabolic pattern of individual bacterial cells. It seems likely that similar variations in susceptibility will occur among bacteria growing in the animal body.

Two independent findings in the present experiments suggest that isoniazid is bactericidal for tubercle bacilli only during autosynthetic activity. First, there was a delay before any significant bactericidal action started; second, terramycin, which inhibited growth without killing the cells, almost totally abolished the bactericidal action of isoniazid. Both of these findings indicate that isoniazid is probably active only against actively growing cells. It was stated in the introduction that streptomycin has similar properties in that it is not bactericidal for the tubercle bacillus in a non-nutrient medium. However, in Dubos-Davis medium, with increasing concentrations of streptomycin, the survival curve becomes increasingly steep and killing finally ensues without any delay (figure 5). This suggests that streptomycin is more direct than isoniazid in its action upon the bacterial cell.

Transferring these findings to conditions in the animal body, neither drug is likely to destroy all bacilli, at least in a short time, even if it is assumed that all are accessible and none resistant. However, the use of two drugs offers some hope of advance. At the end of two days' incubation, 34 per cent of bacterial units exposed to 1.0 γ of streptomycin and 57 per cent of those exposed to 0.1 γ of isoniazid per ml. of Dubos-Davis medium still survived. It may be assumed that the bacilli which were killed were those whose metabolic state rendered them most vulnerable, the survivors being in a less susceptible state. Yet when both drugs acted together in the same concentration and for the same length of time, all but 1.2 per cent of the bacterial units were killed. One hundred times the concentration of isoniazid could not equal this effect, and ten times the concentration of streptomycin was needed to achieve the same effect, so it is clear that the combined action is not simply additive. It may be inferred that streptomycin and isoniazid together exert a bactericidal action, not only upon those bacilli in a given population which are killed by either drug alone, but also upon many bacilli whose metabolic state renders them temporarily refractory to the action of a single drug. By what means the combined action of two drugs can attack such relatively refractory cells cannot usefully be discussed here.

It is an old idea that tubercle bacilli may pass through a resting phase in the animal body. Koch (40) believed them to be present in the form of spores; Jensen has discussed them in relation to the present problem (5). Perhaps some of these can be destroyed by the simultaneous use of two bactericidal antituberculous drugs.

Summary

The survival rate of *M. tuberculosis* H37Rv in Dubos-Davis medium and in isolated mammalian macrophage cultures was determined during exposure to various concentrations of streptomycin, terramycin, and isoniazid, singly and in combination.

The bactericidal activity of streptomycin varies as the drug concentration; that of isoniazid is largely independent of drug concentration. The last two drugs possess comparable bactericidal activities in low concentrations, but terramycin is almost entirely bacteriostatic.

Bacilli potentially resistant to either streptomycin or isoniazid are killed by subinhibitory concentrations of the two drugs acting simultaneously. The combined use of these two drugs results in an acceleration of the death rate of both intracellular and extracellular tubercle bacilli. Terramycin when used with isoniazid abolishes the bactericidal activity of the latter.

To achieve a potentiation of bactericidal activity against intracellular tubercle bacilli by the combined use of streptomycin and isoniazid, relatively high concentrations of streptomycin are necessary because of the limited penetration of streptomycin into cells. Isoniazid is likely to prove more effective than para-aminosalicylic acid in the control of streptomycin-resistant variants because of its capacity to kill intracellular tubercle bacilli.

The experimental data are discussed in relation to the control of drug resistance and the persistence, *in vivo*, of drug-susceptible tubercle bacilli during treatment.

Sumario

La Acción Bactericida del Isoniazid, de la Estreptomicina y de la Terramicina sobre los Bacilos Tuberculosos Extracelulares e Intracelulares

El índice de sobrevivencia del *M. tuberculosis* H37Rv en el medio de Dubos-Davis y en cultivos de macrófagos mamíferos aislados fué determinado durante la exposición a varias concentraciones de estreptomicina, terramicina e isoniazid, ya solos o combinados éstos.

La actividad bactericida de la estreptomicina varía conforme a la concentración de la droga; la del isoniazid es en gran parte independiente de la concentración de la droga. Estas dos drogas poseen comprable actividad bactericida a concentraciones bajas, pero la terramicina es casi absolutamente bacteriostática.

Bacilos potencialmente resistentes ya a la estreptomicina o al isoniazid son destruídos por concentraciones subinhibidoras de las dos drogas obrando simultáneamente. El uso combinado de las dos acelera la muerte de los bacilos tuberculosos tanto intra-como extracelulares. La terramicina, al ser usada junto con el isoniazid, anula la actividad bactericida del último.

Para conseguir una potenciación de la actividad bactericida contra los bacilos tuberculosos intracelulares con el uso combinado de estreptomicina e isoniazid, se necesitan concentraciones relativamente altas de estreptomicina, debido a la limitada penetración de esta droga en las células. Es probable que el isoniazid resulte más eficaz que el ácido para-

aminosalicílico en el dominio de las variantes estreptomicinorresistentes por virtud de su capicidad para matar bacilos tuberculosos intracelulares.

Discútense los datos experimentales en relación con el dominio de la farmacorresistencia y la persistencia, *in vivo*, de bacilos tuberculosos farmacosusceptibles durante el tratamiento.

Resume

L'action bactéricide de l'isoniazide, de la streptomycine et de la terramycine sur les bacilles tuberculeux extracellulaires et intracellulaires

Le taux de survivance du *M. tuberculosis* H37Rv, en milieu Dubos-Davis et en cultures isolées de macrophages de mammifères, a été déterminé au cours de l'exposition à des concentrations variées de streptomycine, de terramycine et d'isoniazide, isolément ou en association.

L'activité bactéricide de la streptomycine varie avec la concentration de la substance utilisée; celle de l'isoniazide est largement indépendante de la concentration. Ces deux dernières substances possèdent à faible concentration, un pouvoir bactéricide comparable, mais la terramycine est presque entièrement bactériostatique.

Les bacilles disposant d'un potentiel de résistance soit pour la streptomycine, soit pour l'isoniazide, sont tués par des concentrations sub-inhibitrices des deux antibiotiques agissant simultanément. L'usage combiné de ces deux médicaments a pour résultat l'accélération de la mortalité des bacilles intra et extracellulaires. La terramycine, utilisée avec l'isoniazide abolit le pouvoir bactéricide de celle-ci.

Pour disposer du potentiel d'action bactéricide contre les bacilles tuberculeux intracellulaires par l'emploi combiné de la streptomycine et de l'isoniazide, il est nécessaire d'utiliser des concentrations relativement élevées de streptomycine, en raison de la pénétration limitée de la streptomycine dans les cellules. L'isoniazide est susceptible de se montrer plus efficace que l'acide para-aminosalicylique pour juguler les variétés streptomycinorésistantes, en raison de sa capacité de tuer les bacilles intracellulaires.

Les données expérimentales relatives au contrôle de la résistance et de la persistance, *in vivo*, de bacilles sensibles à une drogue pendant le traitement, sont discutées.

Acknowledgments

We wish to thank Professor H. W. Florey for his interest and suggestions during the course of this work, Mr. A. A. Argyle and Mr. D. Etheridge for technical assistance, and Dr. M. A. Jennings and Dr. H. Harris for criticism and help in the preparation of this paper.

The photomicrographs were taken by Mr. B. H. Glass.

Supplies of isoniazid (Pycazide) were made available through the courtesy of Herts Pharmaceuticals, Ltd., Welwyn Garden City, Hertfordshire, U. K.

REFERENCES

(1) Feldman, W. H., Hinshaw, H. C., and Mann, F. C.: Streptomycin in experimental tuberculosis, Am. Rev. Tuberc., 1945, *52*, 269.

(2) Feldman, W. H., Karlson, A. G., and Hinshaw, H. C.: Streptomycin in experimental tuberculosis: The effects in guinea pigs following infection by intravenous inoculation, Am. Rev. Tuberc., 1947, *56*, 346.

(3) Steenken, W., Jr.: Streptomycin in guinea pig tuberculosis, Am. Rev. Tuberc., 1947, *56*, 382.

(4) Corper, H. J., and Cohn, M. L.: The probable mechanism of streptomycin action in tuberculosis, Yale J. Biol. & Med., 1948–1949, *21*, 181.

(5) Jensen, K. A.: Biological studies on streptomycin therapy, Acta tuberc. Scandinav., 1949, Supplement 21, p. 42.

(6) Steenken, W., Jr., Wolinsky, E., Pratt, P. C., and Smith, M. M.: Streptomycin in guinea pigs with discrete chronic tuberculous lesions, Am. Rev. Tuberc., 1952, *66*, 194.

(7) Steenken, W., Jr., and Wolinsky, E.: Antituberculous properties of hydrazines of isonicotinic acid (Rimifon, Marsilid), Am. Rev. Tuberc., 1952, *65*, 365.

(8) Buck, M., and Schnitzer, R. J.: The development of drug resistance of *M. tuberculosis* to isonicotinic acid hydrazide, Am. Rev. Tuberc., 1952, *65*, 759.

(9) Steenken, W., Jr., Meade, G. M., Wolinsky, E., and Coates, E. O., Jr.: Demonstration of increased drug resistance of tubercle bacilli from patients treated with hydrazines of isonicotinic acid (notes), Am. Rev. Tuberc., 1952, *65*, 754.

(10) Bloch, R. G., Vennesland, K., Ebert, R. H., and Gomori, G.: The effect of streptomycin, para-aminosalicylic acid (PAS), and their combination on the tubercle bacillus *in vitro* and *in vivo*, Am. Rev. Tuberc., 1949, *59*, 554.

(11) Beck, F., and Yegian, D.: A study of the tubercle bacillus in resected pulmonary lesions, Am. Rev. Tuberc., 1952, *66*, 44.

(12) Marshall, E. K., Jr.: The absorption, distribution and excretion of streptomycin, J. Pharmacol., 1948, *92*, 43.

(13) Suter, E.: Multiplication of tubercle bacilli within phagocytes cultivated *in vitro*, and effect of streptomycin and isonicotinic acid hydrazide, Am. Rev. Tuberc., 1952, *65*, 775.

(14) Mackaness, G. B.: The action of drugs on intracellular tubercle bacilli, J. Path. & Bact., 1952, *64*, 429.

(15) Mackaness, G. B., and Smith, N.: The action of isoniazid (isonicotinic acid hydrazide) on intracellular tubercle bacilli, Am. Rev. Tuberc., 1952, *66*, 125.

(16) Tison, F.: Dissociation des pouvoirs "bactériostatique" et "bactéricide" de la streptomycine *in vitro* sur le bacille de Koch, Ann. Inst. Pasteur, 1950, *79*, 100.

(17) Middlebrook, G., and Yegian, D.: Certain effects of streptomycin on *Mycobacteria in vitro*, Am. Rev. Tuberc., 1946, *54*, 553.

(18) Canetti, G.: Le Bacille de Koch. Dans la Lesion Tuberculeuse du Poumon, Editions Medicales Flammarion, France, 1946.

(19) Medlar, E. M., Bernstein, S., and Steward, D. M.: A bacteriologic study of resected tuberculous lesions, Am. Rev. Tuberc., 1952, *66*, 36.

(20) Ilavsky, J.: Synergistic action of isonicotinic acid hydrazide and streptomycin *in vitro*, Am. Rev. Tuberc., 1952, *65*, 777.

(21) Knox, K. R., Maclean, K. S., and Robson, J. M.: New results with isonicotinic acid hydrazide, Brit. M. J., 1952, *1*, 1081.

(22) Middlebrook, G.: Sterilization of tubercle bacilli by isonicotinic acid hydrazide and the incidence of variants resistant to the drug *in vitro*, Am. Rev. Tuberc., 1952, *65*, 765.

(23) Dubos, R. J., and Davis, B. D.: Factors affecting the growth of tubercle bacilli in liquid media, J. Exper. Med., 1946, *83*, 409.

(24) Fenner, F., Martin, S. P., and Pierce, C. H.: The enumeration of viable tubercle bacilli in cultures and infected tissues, Ann. New York Acad. Sc., 1949, *52*, 751.

(25) Fenner, F.: The enumeration of viable tubercle bacilli by surface plate counts, Am. Rev. Tuberc., 1951, *64*, 353.

(26) Hanks, J. H.: The longevity of chick tissue cultures without renewal of medium, J. Cell & Comp. Physiol., 1948, *31*, 235.

(27) Garrod, L. P.: The nature of the action of streptomycin on tubercle bacilli, Am. Rev. Tuberc., 1950, *62*, 582.

(28) Eagle, H., Fleischman, R., and Levy, M.: Development of increased resistance to antibiotics: 1. Continuous spectrum of resistance to penicillin, chloramphenicol and streptomycin, J. Bact., 1952, *63*, 623.

(29) Szybalski, W., and Bryson, V.: Bacterial resistance studies with derivatives of isonicotinic acid hydrazide, Am. Rev. Tuberc., 1952, *65*, 768.

(30) Jawetz, E., Gunnison, J. B., and Speck, R. S.: Studies on antibiotic synergism and antagonism: The interference of aureomycin, chloramphenicol and terramycin with the action of streptomycin, Am. J. M. Sc., 1951, *222*, 404.

(31) Gunnison, J. B., Coleman, V. R., and Jawetz, E.: Interference of aureomycin and terramycin with action of penicillin *in vitro*, Proc. Soc. Exper. Biol. & Med., 1950, *75*, 549.
(32) Karlson, A. G., and Ikemi, Y.: The occurrence of human-type tubercle bacilli (H37Rv) resistant to isonicotinic acid hydrazide in a culture not previously exposed to the drug, Proc. Staff Meet. Mayo Clin., 1952, *27*, 239.
(33) Demerec, M.: Origin of bacterial resistance to antibiotics, J. Bact., 1948, *56*, 63.
(34) Hsie, J., and Bryson, V.: Genetic studies on the development of resistance to neomycin and dihydrostreptomycin in *Mycobacterium ranae*, Am. Rev. Tuberc., 1950, *62*, 286.
(35) Witkins, E. M.: Nuclear segregation and the delayed appearance of induced mutants in *Escherichia coli*, Cold Springs Harbor Symp. Quant. Biol., 1951, *16*, 357.
(36) Jawetz, E., Gunnison, J. B., Bruff, J. B., and Coleman, V. R.: Studies on antibiotic synergism and antagonism: Synergism among seven antibiotics against various bacteria *in vitro*, J. Bact., 1952, *64*, 29.
(37) Schnitzer, R. J., Lafferty, L. C., and Buck, M.: The role of antibodies in experimental drug resistance of *Trypanosoma equiperdum*, J. Immunol., 1946, *54*, 47.
(38) Price, P. B.: The meaning of bacteriostasis, bactericidal effect, and rate of disinfection, Ann. New York Acad. Sc., 1950, *53*, 76.
(39) Hinshelwood, C.: Decline and death of bacterial populations, Nature, 1951, *167*, 666.
(40) Koch, R.: Die Aetiologie der Tuberkulose, Mitt. kais. Gesundheitsamte, 1884, *2*, 1.

IRRADIATED ANTITUBERCULOSIS VACCINE, INCLUDING COMPARISON WITH BCG IN EXPERIMENTALLY INFECTED GUINEA PIGS[1]

JOSEPH B. SEAGLE, ALFRED G. KARLSON, AND WILLIAM H. FELDMAN[2]

(Received for publication September 5, 1952)

INTRODUCTION

The search for a method by which dependable and prolonged immunity might be produced in man and animals against tuberculosis has attracted the attention of many since the discovery of the tubercle bacillus by Robert Koch in 1882. Through the years numerous agents have been acclaimed as showing promise of fulfilling the many criteria needed to be met by a vaccine against tuberculosis. Thus far, however, BCG has been the only antituberculosis vaccine to have gained wide recognition and, although it is being used in large areas of the world today, its value has not been accepted by all physicians. Recently, reports (1–3) have been made of successful attempts to confer resistance against experimental tuberculosis on guinea pigs and mice by multiple intraperitoneal and subcutaneous injections of nonviable, ultraviolet light-irradiated, human-type tubercle bacilli. Accordingly, the present writers were encouraged to attempt to evaluate the protective potential of such an irradiated vaccine when administered to guinea pigs by clinically acceptable routes.

In 1906, Di Donna (4) exposed cultures of tubercle bacilli to direct sunlight. When he vaccinated his animals with several injections of viable tubercle bacilli attenuated by sunlight, he claimed to have protected guinea pigs against an infection which killed control animals. Resistance to reinfection with tubercle bacilli in guinea pigs also was reported by Bisceglie (5) in 1926 after vaccination of the animals with human-type tubercle bacilli previously attenuated with the rays of radium. In 1939, Smithburn and Lavin (6) reported, like Di Donna, that irradiated viable organisms possessed the capacity of inducing demonstrable immunity in guinea pigs but that organisms killed by radiation did not induce measurable resistance. It was realized by Smithburn and Lavin, however, that heavy suspensions of organisms were killed only after relatively long periods of irradiation and that, when less dense suspensions of organisms were irradiated, a shorter exposure to ultraviolet light would suffice to kill all of the organisms. These workers pointed out that it might be possible to irradiate a suspension of lesser density than they had used so as to render the organisms nonviable while still maintaining the antigenicity of the bacilli.

In 1947, Olson, Habel, and Piggott (1), of the Public Health Service, reported an experiment in which human-type tubercle bacilli, killed by ultraviolet light,

[1] Abridgement of portion of thesis submitted by Dr. Seagle to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Pediatrics.

[2] From the Mayo Foundation, University of Minnesota, Rochester, Minnesota.

were employed as an immunizing agent. These writers found resistance in their vaccinated guinea pigs which was based on mortality statistics only; the resistance was equal to that obtained in control animals vaccinated with BCG and was greatly superior to that in nonvaccinated control guinea pigs. This study was followed in 1950 by similar experiments conducted by Sarber, Nungester, and Stimpert (2). These investigators confirmed the findings of Olson's group and conducted further studies with vaccinated guinea pigs. The guinea pigs were infected intraperitoneally with 0.1 or 0.01 mg. of the virulent organisms given either three or four weeks after the last of the three intraperitoneal injections of the irradiated vaccine. At the termination of the study, protection was noted when the organs of predilection were examined at necropsy and were compared with those of nonvaccinated controls. Milzer, Levinson, and Lewis (3), in 1950, reported attempts to immunize mice against experimental tuberculosis. The animals received four weekly subcutaneous injections of a nonviable vaccine prepared by exposing suspensions of a virulent human strain (H37Rv) of tubercle bacilli to ultraviolet light. The results were considered to be comparable to those observed in a control group of mice vaccinated with BCG.

Birkhaug and Darricarrere (7) reported more recently the results of similar studies with ultraviolet light-killed tubercle bacilli (strains H37Rv and 199RB) in which they vaccinated groups of guinea pigs intraperitoneally with irradiated tubercle bacilli and with BCG. They found that BCG showed antigenic potency superior to irradiated tubercle bacilli, as indicated by greater hypersensitivity to tuberculin and greater resistance against a subsequent virulent inoculation.

MATERIALS AND METHODS

Two separate experiments were undertaken in an attempt to determine whether resistance could be conferred on guinea pigs by a single injection of a vaccine containing tubercle bacilli which had been killed by ultraviolet light. In the first experiment, the extent of tuberculosis found in infected animals which had been vaccinated with the nonviable irradiated vaccine, both by intracutaneous injection with a syringe and by the multiple puncture (disc) method of Rosenthal (8), was compared with the extent of tuberculosis found in nonvaccinated and BCG-vaccinated control animals. In the second experiment, the irradiated vaccine was injected by syringe into separate groups of guinea pigs by the intracutaneous, subcutaneous, intramuscular, and intravenous routes before these and nonvaccinated control animals were infected.

The vaccine containing the ultraviolet light-killed bacilli which was used in both experiments was obtained from the Parke, Davis & Company laboratories.[3] The vaccine was prepared in the manner described by Sarber, Nungester, and Stimpert (2). The irradiated vaccine received for use in this study contained 1.0 mg. of organisms per 1.0 ml. of suspension.[4] The suspension was then concentrated by centrifugation to approximately fifteen times the original strength. The BCG used in comparative immunogenic studies in the first experi-

[3] Supplied through the kindness of Dr. F. D. Stimpert.

[4] Designated Lot No. 8 by Parke, Davis & Company Laboratories, Detroit, Michigan.

ment was obtained through the courtesy of Dr. S. R. Rosenthal of the Tice Laboratories of the Chicago Municipal Tuberculosis Sanitarium. This contained 15.0 mg. of organisms per 1.0 ml.[5]

OBSERVATIONS

Tests of viability: In order to establish further the alleged nonviability of the tubercle bacilli contained in the irradiated vaccine, 6 healthy, tuberculin-negative guinea pigs were injected intravenously with 0.1 ml. (approximately 1.5 mg.) of the irradiated vaccine. Five additional healthy tuberculin-negative guinea pigs were given 0.1 ml. (1.5 mg.) of BCG intravenously. None of these 11 animals was subsequently infected with virulent tubercle bacilli. One of the 5 animals which received BCG intravenously died after approximately five months. No evidence of active tuberculosis was found at necropsy of this animal, nor was there evidence of tuberculosis in the spleen, lungs, liver, or tracheobronchial lymph nodes when these organs were studied microscopically. The remaining 10 animals were killed for necropsy approximately seven months after injection of the vaccines, at which time no evidence of active tuberculosis was discovered grossly. The spleen of each of the 6 animals which received only the irradiated vaccine was cultured when necropsy was performed. This was done with the realization that positive cultures would be of questionable significance since the animals had been housed in the same building (although in separate cages) with the infected animals of the study. It was believed, however, that negative cultures of the spleens at necropsy would serve as further evidence against the possibility of viability of any of the irradiated bacilli. Consequently, cultures were made from the spleens of these 6 guinea pigs which had been inoculated with only the irradiated vaccine. In the culture from one of these spleens several colonies were found which morphologically were identified as those of *Mycobacterium tuberculosis*. The pathogenicity of the organisms was not proved. These colonies contained acid-fast bacillary forms. The lungs and the tracheobronchial lymph nodes of this animal gave histologic evidence of active tuberculosis, and acid-fast bacilli were found in small necrotic abscesses of the lungs. Cultures of the spleens of the other animals in this group failed to yield growth, and the gross and histologic studies of the organs of predilection revealed no evidence of tuberculosis. Four egg-yolk–agar slants were seeded heavily with the irradiated vaccine. These cultures gave no evidence of growth after four months of incubation at 37°C. The significance of these observations on the viability of the bacterial cells in the irradiated vaccine will be discussed subsequently.

Intracutaneous Vaccination

Eighty-one healthy, male, tuberculin-negative guinea pigs, the majority weighing 810 to 860 gm., were divided into four groups as follows:

Group 1—23 guinea pigs: These animals were designated as nonvaccinated controls.

Group 2—21 guinea pigs: Vaccinated intracutaneously with 0.1 ml. (1.5 mg.) BCG by the multiple puncture (disc) method described by Rosenthal (8).

[5] Dated October 5, 1950, and designated Lot No. 902L.

Group 3—22 guinea pigs: Vaccinated intracutaneously with 0.1 ml. (1.5 mg.) irradiated vaccine by the multiple puncture (disc) method.

Group 4—15 guinea pigs: Vaccinated intracutaneously with 0.1 ml. (1.5 mg.) irradiated vaccine by injection with a tuberculin syringe fitted with a 25-gauge needle.

Thirty-two days after the vaccination of *Groups 2, 3, and 4*, the animals in all four groups were again tuberculin tested with 0.1 ml. of a 1:100 dilution of Old Tuberculin. Thirty-nine days after vaccination each animal in the four groups was infected subcutaneously over the sternum with 0.001 mg. of a fifteen-day-old culture of *M. tuberculosis* var. *hominis*, strain H37Rv, of standard virulence for guinea pigs.

The experiment was terminated 166 days after challenge when approximately half of the unvaccinated controls had died. The animals still surviving at this time in all four groups were killed and subjected to necropsy.

TABLE 1

REACTIONS TO 0.1 MILLILITER OF A 1:100 DILUTION OF OLD TUBERCULIN IN THE TWO EXPERIMENTS AFTER VACCINATION AND BEFORE THE ANIMALS WERE INFECTED

	GROUP	TOTAL ANIMALS	DAYS AFTER VACCINATION WHEN TUBERCULIN TESTED	NEGATIVE REACTIONS	EQUIVOCAL REACTIONS	DEFINITELY POSITIVE REACTIONS
Experiment 1		81				
Nonvaccinated controls	1	23		23	0	0
BCG-vaccinated controls	2	21	32	0	0	21
Irradiated vaccine—intracutaneously (disc)	3	22	32	6	13	3
Irradiated vaccine—intracutaneously (syringe)	4	15	32	3	12	0
Experiment 2		70				
Nonvaccinated controls	1	14		14	0	0
Irradiated vaccine—intracutaneously	2	14	40	1	13	0
Irradiated vaccine—subcutaneously	3	14	40	10	4	0
Irradiated vaccine—intramuscularly	4	14	40	7	7	0
Irradiated vaccine—intravenously	5	14	40	11	3	0

Of the 81 animals in the four groups, 2 died twenty-four and forty-three days, respectively, after they had been infected. These animals were considered to have died prematurely since the death of neither of them could be attributed to tuberculosis when necropsy was performed. These 2 guinea pigs were not considered further in the experiment.

Local reactions to vaccination: Local reactions of all of the vaccinated animals were mild, the greatest reactions observed being papules of a diameter of 2 to 6 mm. at the sites of vaccination of the animals in *Group 4*. In no case did gross abscesses, draining sinuses, or palpable axillary lymphadenopathy develop following vaccination and before inoculation of the animals with virulent tubercle bacilli.

Tuberculin tests after vaccination: The tuberculin reactions obtained in the study are summarized in table 1. The BCG-vaccinated animals in *Group 2* all

reacted with strongly positive tuberculin reactions. Of the 22 animals vaccinated with the irradiated vaccine in *Group 3*, only 3 reacted with definitely positive tuberculin tests of 10.0 mm. or more of induration and erythema plus necrosis. Six of the remaining animals in *Group 3* were tuberculin negative, and the remaining 13 animals in this group reacted with slight thickening and erythema of the skin (5 to 9 mm. in diameter) at the site of testing. The reaction observed, while differing greatly from the reactions classified as "tuberculin negative" in the nonvaccinated animals, could not be considered to be a positive reaction of the character usually observed in hypersensitive tuberculous guinea pigs. These 13 animals were classified as having equivocal reactions to tuberculin. Of the 15 animals in *Group 4* which had received the irradiated vaccine intracutaneously by syringe, 3 had negative tuberculin reactions, and in 12 the reactions were equivocal, consisting of 5 to 9 mm. areas of thickening of the skin. None of the latter,

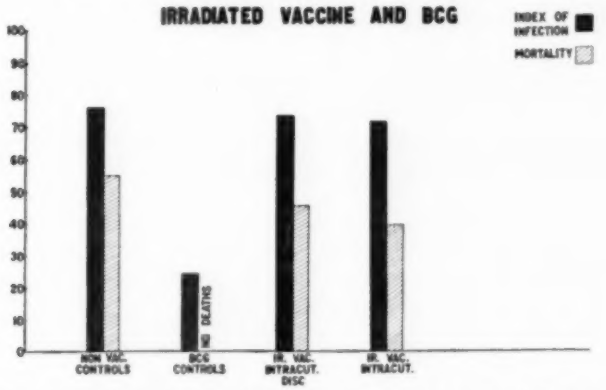


FIG. 1. The index of infection based on microscopic examination of the organs of predilection is represented by the black columns. The theoretical maximal degree of infection is 100. The shaded columns represent the mortality (per cent) in each group of animals at the termination of the experiment.

however, demonstrated definitely positive reactions or necrosis. All of the animals in the first experiment were tuberculin positive when tested 164 days after being infected with virulent tubercle bacilli.

Mortality and gross and microscopic pathologic changes: When the experiment was terminated, 54.5 per cent of the nonvaccinated control animals had died of tuberculosis. At this time none of the BCG-vaccinated animals was dead, whereas 10 of the 22 animals in the group which had received the irradiated vaccine (disc) and 6 of the 15 animals which had received the irradiated vaccine intracutaneously by syringe were dead. The mortality for the three vaccinated groups was 0, 45.4, and 40.0 per cent, respectively, as may be seen in figure 1.

The spleens, lungs, livers, tracheobronchial lymph nodes, and the sites of injection of the infecting organisms of all the animals were studied histologically. The results of these observations were tabulated to show the numerical index of infection (9) and the results are portrayed graphically in figure 1.

FIG. 2. Schematic presentation of the extent of tuberculosis seen at necropsy in the first experiment. The rectangle and oval represent the liver and the spleen, respectively. The large dot or circle in the arrow represents an abscess or ulcer at the site of inoculation. Miliary and nodular lesions are indicated by small and large dots. Diffuse involvement of an organ is indicated by complete blackening. The numerals show the number of days of infection and the black bar indicates that the animal died before the experiment was completed.

The theoretical maximal amount of disease possible in this index of infection is 100. The indices for the respective groups were as follows: nonvaccinated controls, 75.5; BCG controls, 24.7; irradiated vaccine (disc), 73.0; irradiated vaccine (syringe), 71.0. The extent and severity of the infection in the nonvaccinated control animals, and in those which received irradiated vaccine, were indistinguishable microscopically; all revealed large, active tuberculous processes. Caseation and liquefaction necrosis were widespread, although in some sections there were evidences of healing in the form of fibrosis and hard tubercles. In contrast to the foregoing, the animals vaccinated with BCG prior to infection were found to have much less extensive disease. Frequently, the major organs were entirely free of demonstrable disease and even the tracheobronchial lymph nodes of a few animals were uninvolved by the tuberculous process. In this group, fibrosis and evidences of inactive tuberculosis were much more prominent than in the other three groups of animals, although in a few sections caseation and necrosis were observed.

The extent of the tuberculous lesions found at necropsy is represented schematically in figure 2. It was noted that the gross tuberculous lesions found in the animals which had received BCG prior to infection with virulent tubercle bacilli were much less extensive than those found in the animals of the other three groups. In approximately half of the BCG-vaccinated animals, gross tuberculous lesions were minimal or absent in the spleen, lungs, and liver. In a few of these animals, large tuberculous foci in these organs, with or without necrosis, had developed. In contrast to this, it was found that in the nonvaccinated control guinea pigs, as well as in the groups which had received irradiated vaccine, extensive tuberculous foci and abscesses in the majority of the organs of predilection had developed.

Vaccination by One of Four Parenteral Routes

The objective of this experiment was to compare the immunogenic effects of the ultraviolet light-irradiated vaccine when administered in the same dosage by different parenteral routes.

Seventy healthy, male, tuberculin-negative guinea pigs with an average weight of 840 gm. were divided into five groups of 14 animals each. One group was composed of guinea pigs designated as nonvaccinated control animals; and each of the animals in the other four groups received, respectively, 0.1 ml. (approximately 1.5 mg.) of the irradiated bacilli. One group received the vaccine intracutaneously, one subcutaneously, one intramuscularly, and one intravenously. No animals in this experiment were vaccinated with BCG. The 70 animals were tuberculin tested the second time forty days after vaccination. Two days later each of the guinea pigs in the five groups was inoculated subcutaneously over the sternum with 0.001 mg. of a laboratory culture of virulent human-type tubercle bacilli (H37Rv) of standard virulence for guinea pigs.

The experiment was terminated 133 days after all of the animals had been infected, at which time half of the nonvaccinated control animals had died. All of the animals surviving at this time were killed and subjected to macroscopic and microscopic examination of the organs of predilection. None of the guinea pigs died of any cause other than tuberculosis before termination of the experiment.

Local reactions to vaccination: Erythematous papules of a diameter of 3 to 5 mm. were present at the sites of intracutaneous vaccination within three days, and persisted for approximately six weeks. Slight erythema was discernible at the sites of subcutaneous vaccination for two to three days after vaccination. In animals of the groups in which vaccination took place by the intramuscular or intravenous route, no noticeable reaction developed; and in the animals of none of the groups were abscesses, sinuses, or regional lymphadenopathy observed.

Tuberculin tests after vaccination: As has been mentioned, each of the animals in the five groups was tuberculin tested forty days after those in four of the groups had been vaccinated. One-tenth of a milliliter of a 1:100 dilution of Old Tuberculin was used for tuberculin testing, as in the previous experiment. There were no definitely positive reactions to tuberculin among the 56 vaccinated ani-

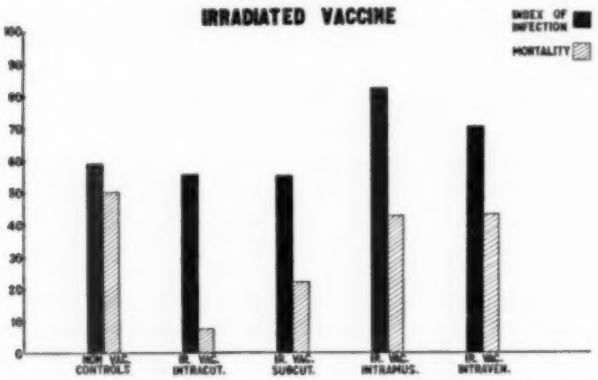


FIG. 3. The results of the microscopic examination of the organs of predilection as expressed by the index of infection are represented by the black columns. The theoretica maximal degree of infection is 100. The mortality (per cent) of the five groups of guines pigs at the end of the experiment is illustrated by the shaded columns.

mals, and all of the nonvaccinated guinea pigs were still tuberculin negative as anticipated. However, of the 14 animals in each of the four groups which had received the irradiated vaccine by either the intracutaneous, subcutaneous, intramuscular, or intravenous routes, there were 13, 4, 7, and 3 animals, respectively, in which slight thickening and erythema of the skin developed forty-eight hours after the injection of tuberculin (table 1). These reactions were similar to the equivocal tuberculin reactions described in the report of the first experiment. In no instance was there found the usual induration or necrosis of the skin such as is characteristically demonstrated in tuberculous guinea pigs following intracutaneous injection of tuberculin. When the 70 animals were again tuberculin tested after infection and two days before the termination of the experiment, all demonstrated typical positive reactions.

Mortality and quantitative pathologic findings: The percentage of guinea pigs which were dead in each of the vaccinated groups when 50 per cent of the non-

vaccinated control animals had died is shown in figure 3. Only 7.1 per cent of the group which had received the vaccine intracutaneously had died, whereas the mortality in the other groups was: subcutaneous vaccination, 21.4 per cent; intramuscular vaccination, 42.8 per cent; and intravenous vaccination, 42.8 per cent.

The index of infection (9) based on microscopic examination of the organs of predilection was not significantly higher in the nonvaccinated control animals than in any of the vaccinated groups of guinea pigs and was even lower than the

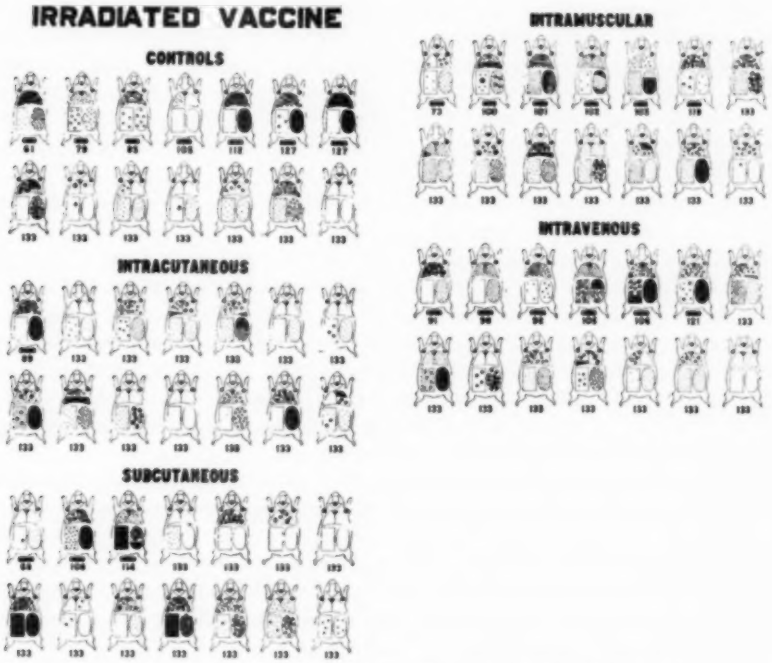


FIG. 4. Schematic illustration of the gross tuberculous lesions found at necropsy in the second experiment. The symbols are the same as those described in the legend of figure 2.

value obtained in two vaccinated groups. The index of infection based on a theoretical maximum of 100 for the five groups of animals was: nonvaccinated controls, 58.1; intracutaneous vaccination, 56.4; subcutaneous vaccination, 55.0; intramuscular vaccination, 81.9; and intravenous vaccination, 69.6 (figure 3).

As would be anticipated from these values, the histologic studies in this experiment revealed progressive tuberculous lesions in all groups regardless of whether or not the animals received the vaccine. The explanation of the relatively high index of infection and the concomitantly more extensive and active microscopic lesions in the group which had received the irradiated vaccine intramuscularly

before infection is obscure. As in the previous experiment, there was occasional evidence of fibrosis and healing in all of the groups of animals.

The extent of the gross tuberculous lesions found at necropsy is indicated in figure 4. As may be noted, there are no significant differences in the macroscopic lesions of the five groups of animals.

DISCUSSION

In the present study the irradiated antituberculosis vaccine was administered in a single injection by a clinically acceptable route. This was considered advisable since the previously reported successful results (1–3) with such a vaccine had all involved administration of the vaccine by multiple injections and in only one previous study had the vaccine been given by a route other than intraperitoneal. The present writers were further encouraged to employ a single injection of the vaccine by a preliminary unpublished report of apparently satisfactory immunity imparted to guinea pigs by a single intraperitoneal injection of 5.0 mg. of the irradiated vaccine.[6]

As has been indicated in the tabulated results of the present study, there was no significant difference between the nonvaccinated control animals and the animals given the irradiated vaccine before infection with respect to the extent of tuberculosis found at necropsy or the results of microscopic study of the spleen, lungs, liver, and tracheobronchial lymph nodes. The relatively low mortality rate (7.1 per cent) of the animals which received the irradiated vaccine by the intracutaneous route in the second experiment was not in agreement with the results when vaccine was similarly used in the first experiment. The relatively low mortality rate also was not reflected in the comparative pathologic examinations of the five groups of animals in the second experiment.

There was a striking increase in the survival times of the animals vaccinated with BCG over those of the control group and the group which received irradiated vaccine in the first experiment. Moreover, the gross examination of these animals at necropsy, as well as the microscopic examination of the tissues, revealed significantly less extensive tuberculosis in the BCG-vaccinated animals as compared with the nonvaccinated animals and the animals in both experiments which received the irradiated vaccine.

The reason or reasons for this failure of the irradiated vaccine to confer any recognizable resistance on the animals after the apparent success of other workers with the same or similar vaccines cannot be stated with certainty at this time. However, many experimental factors may have contributed to the lack of effectiveness of the vaccine in the present study. The experimental factors include, not only the route of administration and the total number of injections of the vaccine as mentioned above, but also the method employed to infect the animals after vaccination and the number of virulent tubercle bacilli contained in the infective inoculum. It should also be emphasized that the total number of nonviable organisms used to vaccinate the animals in these experiments was smaller than

[6] Personal communication to Dr. W. H. Feldman from the Research Department of Parke, Davis & Company.

the dosage used by previous workers. Since reactions to injection of the irradiated vaccine are extremely mild, it would appear advisable in future studies to utilize more than the 1.5 mg. of nonviable tubercle bacilli which were used in the present study.

Perhaps the greatest theoretical advantage of any nonviable vaccine is the elimination of the theoretical possibility of dissociation of the organisms to a virulent state. Great care must be utilized, however, to assure that all of the organisms in the vaccine are in fact dead. Of the 6 animals in this study which, in tests of viability, were injected only with the irradiated vaccine, one was found seven months later to have active caseous pulmonary lesions which contained acid-fast bacilli. Acid-fast organisms were also cultured from the spleen of this animal at necropsy. As was pointed out previously, the possibility exists that this infection could have been contracted from the animal's environment, although such cross-contamination is almost nonexistent in these laboratories. Information has been obtained which indicates that active tuberculous lesions have occurred in the guinea pigs of another worker following injection of the supposedly nonviable irradiated tubercle bacilli from the same lot that was used in this study. This should serve to emphasize the great importance of not using such a vaccine clinically until sufficient safeguards have been met to assure that all of the organisms are dead.

The significance of the tuberculin reactions in these animals following infection of the nonviable bacilli is unknown. As stated previously, all of the BCG-vaccinated guinea pigs developed strongly positive tuberculin reactions prior to infection, while more than half of those which received the irradiated vaccine demonstrated equivocal reactions to tuberculin. None of the nonvaccinated normal animals reacted to tuberculin with similar reactions and none of the equivocal reactions was of the type characteristically elicited in hypersensitive guinea pigs by Old Tuberculin. Since the results of tuberculin testing of guinea pigs after administration of irradiated vaccine have been reported only once previously (7), it was believed that these reactions should be recorded. Periodic tuberculin testing at intervals after vaccination might shed further light on this subject. It was not possible to demonstrate consistent correlation between the size of the tuberculin reactions after vaccination with the irradiated vaccine and the survival rate prior to termination of the experiments. There also was no correlation between the size of the reaction to tuberculin in these animals after vaccination and the extent of tuberculosis found at necropsy.

SUMMARY

Guinea pigs vaccinated with a nonviable, ultraviolet light-irradiated antituberculosis vaccine by a single intracutaneous, subcutaneous, intramuscular, or intravenous injection in the manner and dosage described fail to reveal any measurable resistance to a subsequent infection with virulent human-type tubercle bacilli when compared with nonvaccinated guinea pigs which receive a similar infection with virulent tubercle bacilli.

Guinea pigs vaccinated with BCG prior to infection show a significantly less

extensive and active form of tuberculosis, as well as a significantly lower mortality rate, than do nonvaccinated animals or animals which receive ultraviolet light-irradiated vaccine.

It is important that a vaccine consisting of nonviable tubercle bacilli be not used clinically until adequate tests have been completed to ensure the safety of the product.

The majority of the guinea pigs vaccinated with the irradiated vaccine do not develop typically positive tuberculin reactions when tested with Old Tuberculin. More than half of these animals demonstrate reactions to tuberculin which are recorded as equivocal.

Reactions to irradiated vaccine at the site of injection and in the contiguous lymph nodes are minimal, and systemic reactions are not observed.

SUMARIO

Vacuna Antituberculosa Irradiada, Comprendiendo una Comparación con BCG en Cobayos Infectados Experimentalmente

Los cobayos vacunados con una vacuna tuberculosa aviable, irradiada con rayos ultravioladós, en una sola inyección intracutánea, subcutánea, intramuscular o endovenosa, en la forma y con la dosis descritas, no revelan la menor resistencia mensurable a una infección subsiguiente con bacilos tuberculosos virulentos de tipo humano, al ser comparados con cobayos no vacunados que reciben una infección semejante con bacilos tuberculosos virulentos.

Los cobayos vacunados con BCG antes de la infección muestran una forma de tuberculosis significativamente menos difusa y activa, e igualmente una mortalidad significativamente menor, que los animales no vacunados o que reciben la vacuna irradiada con luz ultravioleta.

Es importante que no se use clínicamente una vacuna compuesta de bacilos tuberculosos aviables hasta llevar a cabo pruebas adecuadas que garanticen la inocuidad del producto.

La mayoría de los cobayos vacunados con la vacuna irradiada no manifiestan, al ser comprobados con Tuberculina Antigua, reacciones tuberculínicas típicamente positivas. Más de la mitad de esos animales muestran reacciones a la tuberculina que se anotan como equívocas.

Las reacciones a la vacuna irradiada en el sitio de la inyección y en los ganglios linfáticos contiguos son mínimas y no se observan reacciones generales.

RESUME

Vaccin antituberculeux irradié—Comparaison avec le BCG chez le cobaye soumis à l'infection expérimentale

Des cobayes ont été vaccinés avec un vaccin inactivé par les ultra-violets. L'inoculation unique sous-cutanée, intramusculaire ou intraveineuse effectuée selon la méthode et la posologie décrite n'a pas révélé de résistance appréciable à l'inoculation ultérieure effectuée avec des bacilles tuberculeux de type humain, par comparaison avec celle observée chez des cobayes, non vaccinés, soumis à une inoculation similaire avec des bacilles tuberculeux virulents.

Les cobayes vaccinés avec le BCG avant l'infection expérimentale présentent une forme de tuberculose nettement moins extensive et moins active et un taux de mortalité beaucoup plus bas que les animaux non vaccinés, ou les animaux vaccinés avec le vaccin irradié par les ultra-violets.

Il est important de renoncer à utiliser en clinique un vaccin constitué par des bacilles non viables jusqu'au moment où des tests adéquats auront rendu le produit d'un usage sûr.

Dans les tests effectués avec la vieille tuberculine, la majorité des cobayes vaccinés avec ce vaccin irradié présentent ne pas les réactions tuberculiniques positives typiques. Plus de la moitié de ces animaux ont des réactions à la tuberculine qualifiées "douteuses."

Au point d'injection et dans les ganglions lymphatiques contigus les réactions au vaccin irradié sont peu marquées et les réactions générales ne sont pas observées.

REFERENCES

(1) Olson, B. J., Habel, K., and Piggott, W. R.: A comparative study of live and killed vaccines in experimental tuberculosis, Pub. Health Rep., 1947, *62*, 293.

(2) Sarber, R. W., Nungester, W. J., and Stimpert, F. D.: Immunization studies with irradiated tuberculosis vaccines, Am. Rev. Tuberc., 1950, *62*, 418.

(3) Milzer, A., Levinson, S. O., and Lewis, M. B.: Immunization of mice with ultraviolet killed tuberculosis vaccines, Proc. Soc. Exper. Biol. & Med., 1950, *75*, 733.

(4) Di Donna, A.: Untersuchungen über die Immunisierung mit durch das Sonnenlicht abgetöteten oder abgeschwachten Milzbrandund Tuberkelbacillen, Zentralbl. f. Bakt., 1906, *42*, 642.

(5) Bisceglie, V.: Versuche über Tuberkulose-Schutzimpfungen mit lebendigen, durch Radiumbehandlung abgeschwächten Koch-Bazillen, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1926, *49*, 272.

(6) Smithburn, K. C., and Lavin, G. I.: Effects of ultraviolet radiation on tubercle bacilli, Am. Rev. Tuberc., 1939, *39*, 782.

(7) Birkhaug, K., and Darricarrere, R.: Comparative antigenic potency of killed irradiated tubercle bacilli and living BCG vaccine, Proc. Soc. Exper. Biol. & Med., 1952, *79*, 387.

(8) Rosenthal, S. R.: Immunologic aspects of BCG vaccination: A strictly controlled study in infants from tuberculous households, J. Pediat., 1950, *36*, 399.

(9) Feldman, W. H.: A scheme for numerical recording of tuberculous changes in experimentally infected guinea pigs, Am. Rev. Tuberc., 1943, *48*, 248.

CHEMOTHERAPY OF EXPERIMENTAL TUBERCULOSIS[1]

VI. Derivatives of Isoniazid

JACK BERNSTEIN, WILLIAM P. JAMBOR, W. A. LOTT, FELIX PANSY, BERNARD A. STEINBERG, AND HARRY L. YALE

(Received for publication October 28, 1952)

INTRODUCTION

In a previous communication the results of the study of the antituberculous activity of a number of acyl hydrazides were reported and the high activity of isoniazid and several of its derivatives was disclosed (1). Numerous publications concerned with the pharmacology and clinical testing of isoniazid and its isopropyl derivative have now appeared, but no other studies on the correlation of structure with activity in the isonicotinic acid series have been published. In the present report the results of *in vitro* and *in vivo* testing of a number of isoniazid derivatives are presented. In the following paper in this series, the results of the *in vitro* and *in vivo* testing of a number of other heterocyclic acid hydrazides and their derivatives are reported (2). The synthesis of both series of compounds will be reported in a subsequent publication (3).

MATERIALS AND METHODS

The compounds were tested for *in vitro* activity against BCG using a modified Kirchner's medium, and were tested in the standardized *in vivo* test against *M. tuberculosis* var. *bovis*, strain Ravenel (4).

In this test, groups of 10 mice of the CF#1 strain were infected intravenously. The test drug was administered by mixing it with finely ground Rockland mouse pellets and letting the mice eat *ad libitum* for 23 days (21 days post infection). The criteria used for detection of activity against tuberculosis were: (*1*) the increase in T_{50} (time of death of 50 per cent of the mice) between the test mice and the untreated controls; and (*2*) for highly active compounds, the number of mice which survived the test period (35 days). In this standardized infection, the T_{50} of the untreated controls is usually between 17.5 and 20 days. No control mice survived for 25 days. Mice fed 0.375 per cent and 0.75 per cent of para-aminosalicylic acid (PAS) served as drug controls. Para-aminosalicylic acid at the levels given produced approximately a four- and seven-day increase in T_{50}, respectively.

OBSERVATIONS

The results obtained in the testing are recorded in tables 1 through 4. In the tables are listed the structure of the compound tested; the minimum inhibitory concentration *in vitro* for the BCG strain of *M. tuberculosis*; the approximate maximum acceptable level of the drug in the diet; the measured drug intake averaged over the first seven days in the therapeutic test; the corresponding change in T_{50}; and the *isoniazid equivalent*[2] of the compound. For the purpose of complete-

[1] From the Squibb Institute for Medical Research, New Brunswick, New Jersey.

[2] The *isoniazid equivalent* is the calculated ratio of equally effective molar doses of isoniazid to the compound tested, in the range of minimally effective concentration. Differences in *isoniazid equivalent* by a factor of two are not significant.

TABLE 1

ISONIAZID DERIVATIVES: DATA ON THE ANTITUBERCULOUS ACTIVITY OF RING-SUBSTITUTED DERIVATIVES

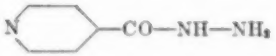
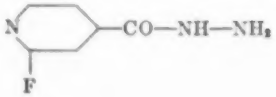
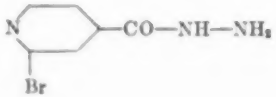
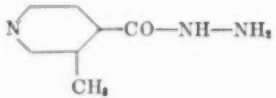
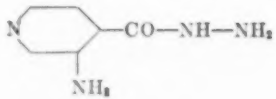
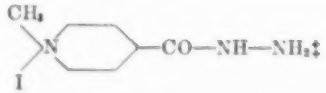
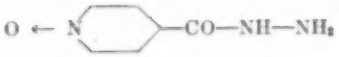
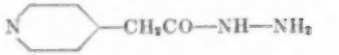
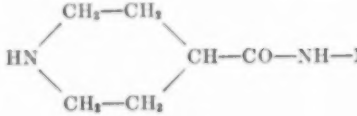
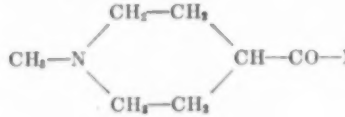
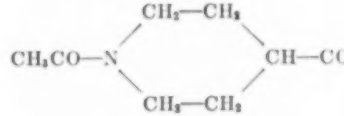
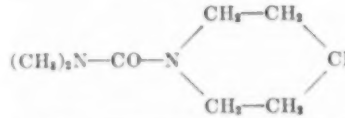
STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{10}°	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	0.02	64	4† 3 3 2	15+ 15+ 8.5 7.0	1
	0.4	64	30 15	0 −0.5	<0.03
	15	64	30 15	1.0 0.5	<0.06
	200	1,000	400 200	0 1.0	<0.003
	1.5	250	100 50	8.0 2.0	0.01
	10	1,000	500 250	−1.0 −3.0	<0.003

TABLE 1.—*Continued*

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	1	500	15 7 3	15+ 10.0 2.0	0.3
	100	1,000	400 200	0 1.0	<0.003
	600	>2,000	1,000 500	0 0	<0.0015
	600	>2,000	750 450	−1.0 0.5	<0.001
	6	500	200 100	−1.5 1.0	<0.008
	600	250	100 50	1.5 0	<0.008

* *See* "Materials and Methods."

† Compounds were tested at one-half, one-quarter, one-eighth, et cetera of the maximum acceptable level in the diet. In this and subsequent tables only the lowest levels that prolonged the T_{50} for more than 15 days are reported. In all cases the higher levels tested gave

TABLE 2

ISONIAZID DERIVATIVES: DATA ON THE ANTITUBERCULOUS ACTIVITY OF VARIOUS ALKYL AND ACYL DERIVATIVES

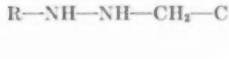
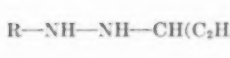
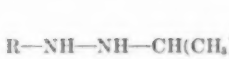
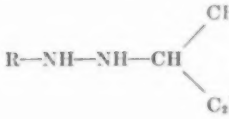
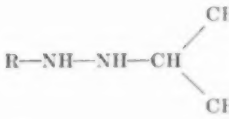
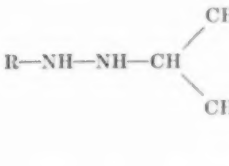
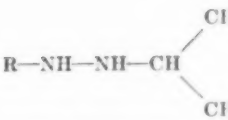
STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	0.1	250	50 30 15	15+ 9.0 0.5	0.1
	3	250	60 30 15	15+ 4.5 0.5	0.1
	0.4	125	8 4 2	15+ 0 −1.5	0.4
	0.4	250	35 15	15+ 15+	0.6+
	5	250	30 15	15+ 0.5	0.15
	4	250	100 60	15+ 15+	0.2+
	5	250	60 30	6.0 2.0	0.06

TABLE 2—*Continued*

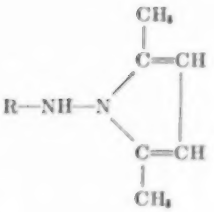
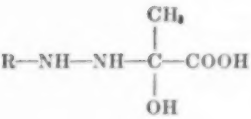
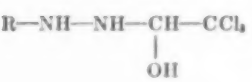
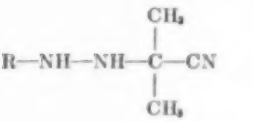
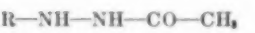
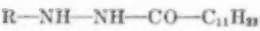
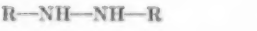
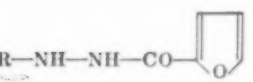
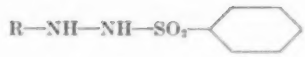
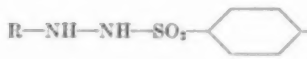
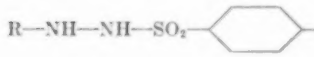
STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_m*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	10	1,000	200 100 50 10	15+ 7.0 4.0 −1.0	0.03
	0.06	500	225 100	15+ 15+	0.08+
	0.04	500	15 7	15+ 5.5	0.6
	0.06	250	30 10 7 4	15+ 14.0 6.0 0	0.4
	3	2,000	450 125 60	15+ 10 0	0.03
	15	1,000	500 225	15+ 12.5	0.02
	0.05	250	30 15	15+ 15+	0.8+
	8	500	100 50	15+ 3	0.02
	15	250	30 10	15+ 1.0	0.15

TABLE 2—*Concluded*

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	3	125	60 30	1.5 1.0	<0.03
	60	2,000	900 450	0 0	<0.004
	600	250	100 60	−0.5 −0.5	<0.03

* *See* "Materials and Methods"

ness, the derivatives of isoniazid which were reported previously (1) have been included in the tables.

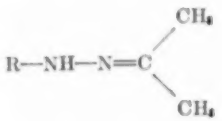
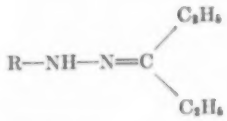
In table 1 are shown the data on the antituberculous activity of a number of ring-substituted derivatives of isoniazid, including piperidine derivatives. (Isoniazid is included for comparison.) The compounds are all less active *in vitro* than isoniazid, and only the hydrazide of isonicotinic acid N-oxide shows appreciable *in vivo* activity, having an *isoniazid equivalent* of 0.3. Ring substitution results in loss of activity, although 3-amino-isonicotinic acid hydrazide does show slight activity (*isoniazid equivalent* of 0.01). Reduction of the pyridine ring to the piperidine ring also results in complete loss of *in vitro* and *in vivo* activity, these compounds having *isoniazid equivalents* of <0.008.

In table 2 are listed the data on the activity of various alkyl and acyl derivatives of isoniazid, the substitution being in the hydrazine fraction of the molecule. These compounds, in general, have low *in vitro* activity, although several of the compounds do inhibit at concentrations below 1 γ per ml. The alkyl derivatives of isoniazid are all active *in vivo*, having *isoniazid equivalents* of 0.08 to 0.6+. The acyl derivatives, with the exception of the benzene sulfonyl derivatives, are also quite active *in vivo*, with *isoniazid equivalents* of 0.02 to 0.8+. In view of the low *in vitro* activity of these compounds, their *in vivo* activity may be accounted for on the basis of their degradation to isoniazid or to a metabolite common to both isoniazid and its derivatives.

In table 3 are listed the data on the activity of the isonicotinyl hydrazones of various aldehydes and ketones. These derivatives appear to be quite active *in vitro* and *in vivo*. The aliphatic and alicyclic mono-carbonyl derivatives, as a class, appear to be most active, having *isoniazid equivalents* of 0.15+ to 0.8. The aromatic derivatives are somewhat less active, with *isoniazid equivalents of* 0.01 to

TABLE 3

DATA ON THE ANTITUBERCULOUS ACTIVITY OF THE ISONICOTINYL HYDRAZONES OF VARIOUS ALDEHYDES AND KETONES

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
R =					
R—NH—N=CH—CH_3	0.04	125	60 30	15+ 15+	0.15+
R—NH—N=CH—$CH(CH_3)_2$	0.1	64	6 4	15+ 4.5	0.4
R—NH—N=CH—C_6H_{11}	0.03	250	100 50	15+ 15+	0.3
R—NH—N=CH—$(CH_2)_7$—CH=CH—CH_3	0.15	250	125 65	15+ 15+	0.15+
R—NH—N=CH—$(CH_2)_8$—CH_3	0.3	250	60 30	15+ 15+	0.3+
	0.02	250	15 7 4	15+ 7.0 0	0.4
	0.02	250	30 15	15+ 15+	0.6+

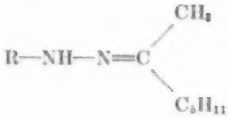
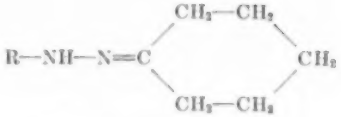
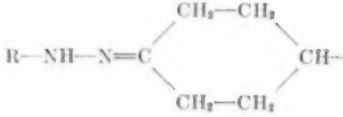
	0.03	250	30 15	15+ 15+	0.6+
	0.006	250	7 3	15+ 7.0	0.8
	0.07	250	30 15	15+ 15+	0.3+
	0.2	250	25 15	15+ 3.5	0.2
	0.05	250	60 30 15	15+ 8.5 0.5	0.2
	0.08	>2,000	450 250 200	15+ 8.0 2.0	0.01
	0.6	500	65 30	15+ 8.0	0.1
	0.08	1,000	125 60	15+ 15+	0.2+

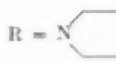
TABLE 3—*Concluded*

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_M*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
R—NH—N=CH—CH=N—NH—R	15	>2,000	1,900	15+	0.01+
			900	15+	
R—NH—N=C(CH$_3$)—CH=N—NH—R	0.15	1,000	35	15+	0.8+
			15	15+	
R—NH—N=C(CH$_3$)—C(CH$_3$)=N—NH—R	0.5	1,000	125	15+	0.2+
			50	15+	
R—NH—N=C(CH$_3$)—CH$_2$—C(=O)—CH$_3$	0.8	1,000	100	15+	0.1+
			50	15+	

* *See* "Materials and Methods"

TABLE 4

DATA ON THE ANTITUBERCULOUS ACTIVITY OF ISONICOTINYL HYDRAZONES OF VARIOUS SUGARS AND STREPTOMYCIN

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_m*	ISONIAZID EQUIVALENT
	γ *per ml.*	*mg. per kg.*	*mg. per kg.*	*days*	
R = 					
R—NH—N=CH(CHOH)$_3$—CH$_2$OH D-ribose	0.05	125	15 7	15+ 3.0	0.3
R—NH—N=CH(CHOH)$_3$—CH$_2$OH L-arabinose	0.02	500	8 7 4	15+ 8.0 3.5	0.6
R—NH—N=CH(CHOH)$_4$—CH$_2$OH D-glucose	0.15	1,000	15 7 3	15+ 4.5 1.0	0.4
R—NH—N=CH(CHOH)$_4$—CH$_2$OH D-galactose	0.015	1,000	15 7 3 2	15+ 5.5 1.5 −0.5	0.4
R—NH—N=C(CH$_2$OH)(CHOH)$_3$—CH$_2$OH D-levulose	0.06	1,000	225 15 7	15+ 2.5 1.0	0.08
R—NH—N=CH—$C_{11}H_{21}O_{10}$ D-maltose	0.15	1,000	10 6	2.5 −1.0	0.1
R—NH—N=CH—C(=R—NH—N)—(CHOH)$_3$—CH$_2$OH D-glucosone	0.04	1,000	7 4	15+ 3.5	1.0
R—NH—N=CH—S†	0.15	2,000	100 25 15 6 6	15+ 14.0 3.5 3.0 1.5	0.4

* *See* "Materials and Methods."

† S—CHO = streptomycin.

0.2+. Activity is also shown by the isonicotinyl hydrazones of aliphatic dicarbonyl compounds which have *isoniazid equivalents* of 0.01+ to 0.8+.

In table 4 are shown the data on the activity of the isonicotinyl hydrazones of various sugars and of streptomycin. These compounds are all active *in vitro* and *in vivo*. The isonicotinyl hydrazones of the pentoses and hexoses have *isoniazid equivalents* of 0.08 to 0.6. Only one disaccharide, D-maltose, was condensed with isoniazid to give a derivative with an *isoniazid equivalent* of 0.1. However, when glucosone was condensed with isoniazid, two molecules of the hydrazide reacted, as expected, and a derivative with an *isoniazid equivalent* of 1.0 was obtained. The isonicotinyl hydrazone of streptomycin was also prepared and tested and showed an *isoniazid equivalent* of 0.4.

SUMMARY

A series of derivatives of isoniazid have been prepared and tested *in vitro* and *in vivo*. The results of the tests permit certain generalizations concerning the relation of structure to antituberculous activity in the isoniazid series.

The introduction of a substituent in the pyridine ring results in loss of *in vitro* and *in vivo* activity.

The reduction of the pyridine ring causes loss of *in vitro* and *in vivo* activity.

Alkylation or acylation of the hydrazine nitrogen produces derivatives which are active *in vivo* although they have low *in vitro* activity.

Condensation of isoniazid with aldehydes or ketones, including sugars, yields derivatives which are active *in vitro* and *in vivo*.

None of the compounds tested are more active on a molar basis than isoniazid.

SUMARIO

Quimioterapia de la Tuberculosis Experimental. VI. Derivados de la Isoniacida

Una serie de derivados de la isoniacida ha sido preparada y ensayada *in vitro* e *in vivo*. Los resultados de esos ensayos permiten hacer ciertas generalizaciones acerca de la relación de la estructura con la actividad.

La introducción de un substituto en el anillo de la piridina hace perder actividad *in vitro* e *in vivo*.

La reducción del anillo de la piridina hace perder actividad *in vitro* e *in vivo*.

La alquil acióno acidilación del nitrógeno de la hidracina produce derivados que son activos *in vivo*, aunque muestran poca actividad *in vitro*.

La condensación de la isoniacida con aldehidos o quetonas, incluso azúcares, rinde derivados que son activos *in vitro* e *in vivo*.

Ninguno de los compuestos ensayados es más activo a base molar que la isoniacida.

RESUME

Chimiothérapie de la tuberculose expérimentale. VI. Dérivés de l'isoniazide

Une série de dérivés de l'isoniazide a été préparée et expérimentée *in vitro* et *in vivo*. Les résultats de ces tests permettent aux auteurs d'en déduire certaines généralisations à l'égard des rapports de la structure et de l'activité.

L'introduction d'un substituant dans le cycle pyridine détermine une perte d'activité *in vitro* et *in vivo*.

La réduction du cycle pyridine détermine la perte de l'activité *in vitro* et *in vivo*.

L'alcoylation ou l'acylation de l'azote de l'hydrazine donne des dérivés actifs *in vivo*, mais dont l'activité *in vitro* est faible.

La condensation de l'isoniazide avec les aldéhydes ou les cétones, y compris les sucres, donne des dérivés actifs *in vitro* et *in vivo*.

Aucun des composés étudiés ne possede une activité molaire supérieure à celle de l'isoniazide.

REFERENCES

(1) Bernstein, J., Lott, W. A., Steinberg, B. A., and Yale, H. L.: Chemotherapy of experimental tuberculosis: V. Isonicotinic acid hydrazide (Nydrazid) and related compounds, Am. Rev. Tuberc., 1952, *65*, 357.

(2) Bernstein, J., Jambor, W. P., Lott, W. A., Pansy, F., Steinberg, B. A., and Yale, H. L.: Chemotherapy of experimental tuberculosis: VII. Heterocyclic acid hydrazides and derivatives, Am. Rev. Tuberc., 1953, 67, 366.

(3) Yale, H. L., Losee, K., Martins, J., Holsing, M., Perry, F. M., and Bernstein, J.: Chemotherapy of experimental tuberculosis: VIII. The synthesis of acid hydrazides, their derivatives and related compounds, J. Am. Chem. Soc. (in press).

(4) Rake, G., Jambor, W., McKee, C. M., Pansy, F., Wiselogle, F. Y., and Donovick, R.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: III. The standardized test, Am. Rev. Tuberc., 1949, *60*, 121.

CHEMOTHERAPY OF EXPERIMENTAL TUBERCULOSIS[1]

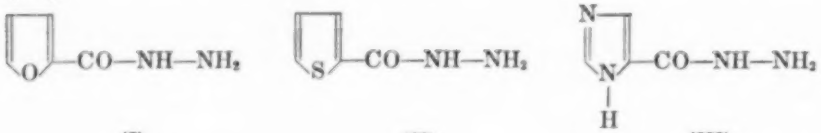
VII. Heterocyclic Acid Hydrazides and Derivatives

JACK BERNSTEIN, WILLIAM P. JAMBOR, W. A. LOTT, FELIX PANSY, BERNARD A. STEINBERG, AND HARRY L. YALE

(Received for publication October 28, 1952)

INTRODUCTION

In the previous paper in this series, the antituberculous activity of isoniazid and a number of its derivatives was reported (1). Further investigation has shown that antituberculous activity is not limited to the isonicotinic acid series and that the hydrazides of several other heterocyclic carboxylic acids are also active in experimental tuberculosis in the mouse. The most active in this new group of antituberculous compounds are 2-furoic acid hydrazide (I), thiophene-2-carboxylic acid hydrazide (II), and imidazole-5-carboxylic acid hydrazide (III).



(I) (II) (III)

A number of derivatives of these active hydrazides have been prepared (2) and the results of the testing form the basis of the present paper.

In addition to these derivatives, the hydrazides of various other heterocyclic carboxylic acids have been synthesized and tested. The heterocyclic acids include derivatives of pyrrole, thiazole, pyrimidine, and pyrazine. Although several of these hydrazides showed slight activity *in vivo*, none of the compounds in this group appears to warrant further investigation.

MATERIALS AND METHODS

The compounds were tested for *in vitro* activity against BCG, using a modified Kirchner's medium and tested in the standardized *in vivo* test against *M. tuberculosis* var. *bovis*, strain Ravenel (3).

In this test, groups of 10 mice of the CF#1 strain were infected intravenously. The test drug was administered by mixing it with finely ground Rockland mouse pellets and letting the mice eat *ad libitum* for 23 days (21 days post infection). The criteria used for detection of activity against tuberculosis were: (*1*) the increase in T_{50} (time of death of 50 per cent of the mice) between the test mice and the untreated controls; and (*2*) for highly active compounds, the number of mice which survived the test period (35 days). In this standardized infection, the T_{50} of the untreated controls is usually between 17.5 and 20 days. No control mice survived for 25 days. Mice fed 0.375 per cent and 0.75 per cent of para-aminosalicylic acid (PAS) served as drug controls. Para-aminosalicylic acid at the levels given produced approximately a four- and seven-day increase in T_{50}, respectively.

[1] From the Squibb Institute for Medical Research, New Brunswick, New Jersey.

TABLE 1

DATA ON THE ANTITUBERCULOUS ACTIVITY OF 2-FUROIC ACID HYDRAZIDE AND ITS DERIVATIVES

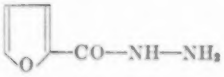
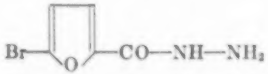
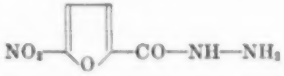
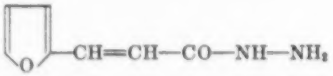
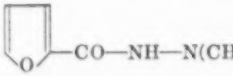
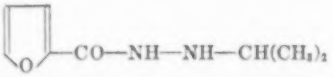
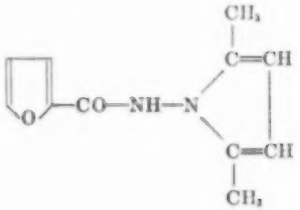
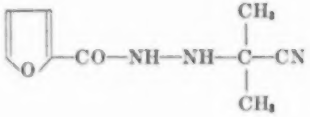
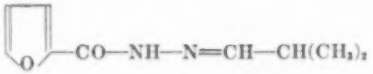
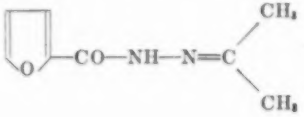
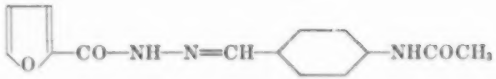
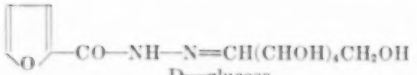
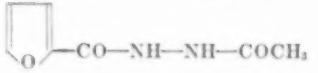
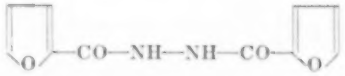
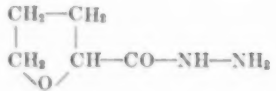
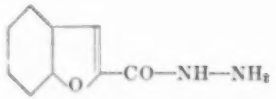
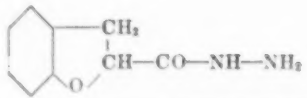
STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	0.06	250	50† 30 10	15+ 15+ −2.0	0.08
	12	125	50 30	−0.5 0.5	<0.04
	0.8	125	50 30	1.0 0.5	<0.02
	6	1,000	400 200	−2.0 −0.5	<0.002
	3	2,000	1,000 450	0.5 0.5	<0.008
	300	500	200 100	−0.5 0.5	<0.004
	15	64	15 8	0 0	<0.1

TABLE 1—*Concluded*

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	15	1,000	450 200	15+ 3.5	0.006
	3	500	200 100	5.0 −1.0	0.01
	0.15	64	30 15	15+ 0	0.1
	4	250	50 30 15	15+ 15+ 1.5	0.1
	4.5	250	100 50	1.5 1.5	<0.02

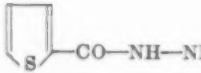
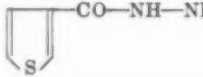
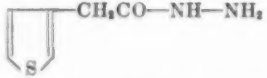
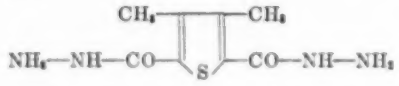
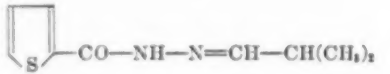
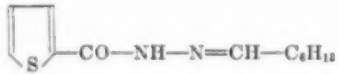
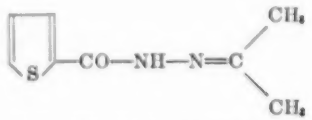
 D—glucose	6	500	125 50 25 15	15+ 4.0 4.0 0.5	0.04
	600	250	100 50	−2.0 2.0	<0.008
	100	250	30 15	15+ −1.0	0.15
	0.3	64	30 15	−1.0 −1.0	<0.04
	2	64	30 15	2.5 0	<0.03
	225	32	15 7	−1.5 2.5	<0.08

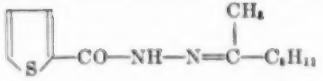
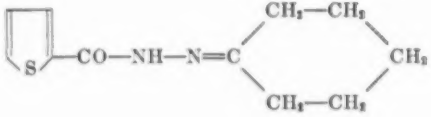
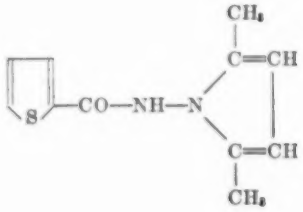
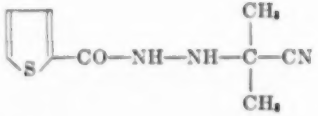
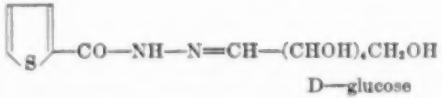
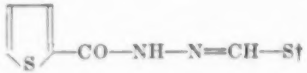
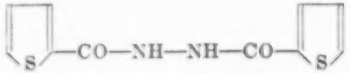
* *See* "Materials and Methods."

† Compounds were tested at one-half, one-quarter, one-eighth, et cetera of the maximum acceptable level in the diet. In this and subsequent tables only the lowest levels that prolonged the T_{50} for more than 15 days are reported. In all cases the higher levels tested gave an increase in T_{50} of at least 15 days.

TABLE 2

DATA ON THE ANTITUBERCULOUS ACTIVITY OF THIOPENE-2-CARBOXYLIC ACID HYDRAZIDE AND RELATED COMPOUNDS

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	8	64	30 10	15+ 2.5	0.08
	8	64	30 15	3.5 1.0	0.03
	100	250	100 50	1.5 0	<0.006
	30	500	200 100	0 2.0	<0.004
	1.2	32	15 7	−2.0 0	<0.15
	0.2-6	125	60 30	15+ 0	0.08
	0.45	64	30 15	1.5 −1.0	<0.04

	3	250	100 70	15+ 10	0.08
	0.15-3	125	60 30	15+ 2.0	0.08
	3-40	1,000	500 200	15+ 4.5	0.01
	3	1,000	400 200	0 −1.0	<0.004
 D—glucose	0.6	1,000	100 50	15+ 1	0.04
	0.25	1,000	250 100 50	15+ 0 0.5	0.08
	15	64	30 15	15+ 0	0.2

* *See* "Materials and Methods." † S-CHO = streptomycin.

TABLE 3

DATA ON THE ANTITUBERCULOUS ACTIVITY OF HYDRAZIDES OF VARIOUS OTHER HETEROCYCLIC CARBOXYLIC ACIDS

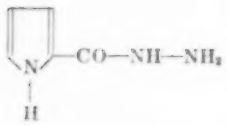
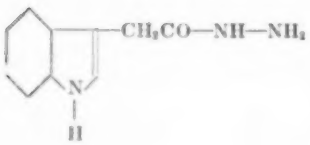
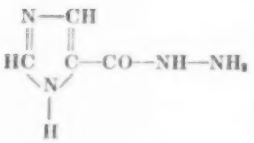
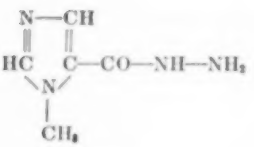
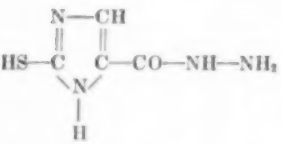
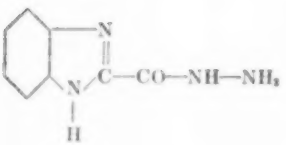
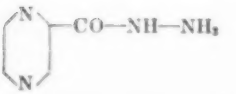
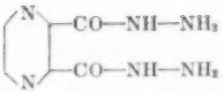
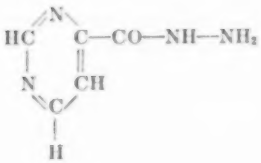
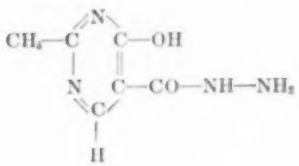
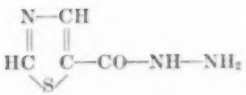
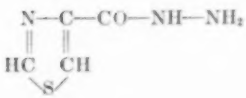
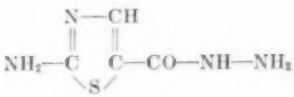
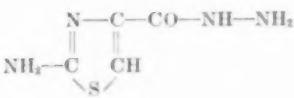
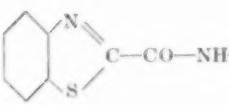
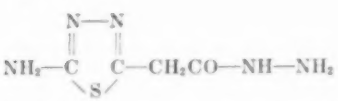
STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}°	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	60	125	50 30	1.0 −1.0	<0.03
	60	500	200 100	−2.0 1.0	<0.004
	2–8	1,000	250 125 60 30	15+ 15+ 6.5 1.0	0.03
	300	500	120 60	0 1.0	<0.01
	250	2,000	800 400	1.0 1.0	<0.02
	15	250	100 50	−2.5 −1.5	<0.01
	1.5	32	15 8	−1.0 0	<0.04

TABLE 3—*Continued*

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG IN VITRO	MAXIMUM ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}*	ISONIAZID EQUIVALENT
	γ per ml.	*mg. per kg.*	*mg. per kg.*	*days*	
	15	125	60 30	0.5 0.5	<0.03
	20	250	100 50	0.5 2.5	<0.006
	450	2,000	1,000 500	0 1.0	<0.001
	10	250	100 50	0.5 2.5	<0.006
	15	64	25 15	0 0	<0.02
	6	500	200 100	14.5 7.5	0.015
	3	250	100 50	7.5 0	0.015
	8	64	30 15	0 −1.0	<0.03
	300	1,000	400	−3.0 −1.0	<0.003

* *See* "Materials and Methods."

OBSERVATIONS

The results which were obtained are recorded in tables 1 through 3. In the tables are listed the structure of the compound tested; the minimum inhibiting concentration for BCG *in vitro*; the approximate maximum acceptable level in the diet; the measured drug intake averaged for the first seven days of the test; the change in T_{50} at that level in the diet; and the *isoniazid equivalent* of the compound.[2]

In table 1 are listed the data for 2-furoic acid hydrazide and its derivatives. 2-Furoic acid hydrazide itself shows high *in vitro* activity and definite *in vivo* activity, having an *isoniazid equivalent* of 0.08. The ring-substituted derivatives of 2-furoic acid hydrazide prepared are inactive *in vivo*, the *isoniazid equivalents* being <0.002 to <0.004. However, several of the derivatives of 2-furoic acid hydrazide, in which substitution is on the hydrazine fraction of the molecule, are active. The active derivatives are those obtained by condensing 2-furoic acid hydrazide with isobutyraldehyde, acetone, acetonylacetone, D-glucose, and furoyl chloride. The *isoniazid equivalents* of these compounds vary from 0.006 to 0.1. Although these derivatives are active *in vivo*, only isobutyraldehyde 2-furoic acid hydrazone shows high *in vitro* activity, and the explanation of the *in vivo* results may be that the compounds are degraded to 2-furoic acid hydrazide or some metabolic product of 2-furoic acid hydrazide. The general observations of the effect of further modification of the isoniazid molecule on activity (1) appear to be true in the 2-furoic acid series. Ring substitution causes loss of activity, reduction of the ring causes loss of activity, and derivatives of the hydrazine fraction of the molecule cause decreased activity. In the 2-furoic acid hydrazide series, this decrease in activity is very pronounced and the derivatives are usually inactive *in vivo*.

In table 2 are shown the data for thiophene-2-carboxylic acid hydrazide and related compounds. In addition to thiophene-2-carboxylic acid hydrazide itself, which has an *isoniazid equivalent* of 0.08, the compounds in this series which show activity are those obtained by the condensation of thiophene-2-carboxylic acid hydrazide with n-heptaldehyde, methyl amyl ketone, cyclohexanone, acetonylacetone, D-glucose, streptomycin, and thiophene-2-carbonyl chloride. These derivatives have *isoniazid equivalents* of 0.01 to 0.2.[3] It is interesting to note that the isomeric thiophene-3-carboxylic acid hydrazide shows only minimal activity, with an *isoniazid equivalent* of 0.03.

In table 3 are shown the results of the tests of hydrazides of various other heterocyclic carboxylic acids. Imidazole-5-carboxylic acid hydrazide is the most active in this group, with an *isoniazid equivalent* of 0.03. Other compounds which show definite *in vivo* activity are 2-aminothiazole-5-carboxylic acid hydrazide

[2] The *isoniazid equivalent* is the calculated ratio of equally effective molar doses of isoniazid to the compound tested, in the range of minimally effective concentration. Differences in *isoniazid equivalent* by a factor of two are not significant.

[3] In both the 2-furoic acid hydrazide series and the thiophene-2-carboxylic acid hydrazide series, only representative condensations were carried out. The reported results are not meant to imply that other related derivatives will not also be active.

(*isoniazid equivalent* of 0.015) and 2-aminothiazole-4-carboxylic acid hydrazide (*isoniazid equivalent* of 0.015). It is especially interesting to note that in the thiazole series the parent compounds are inactive, and the 2-amino substituted derivatives show activity. In the pyridine, furan, and thiophene series, ring substitution results in loss of activity.

SUMMARY

The antituberculous activity of several heterocyclic carboxylic acid hydrazides is reported. The most active compounds in this series are 2-furoic acid hydrazide, thiophene-2-carboxylic acid hydrazide, and imidazole-5-carboxylic acid hydrazide. None of the compounds tested is as active as isoniazid.

SUMARIO

Quimioterapia de la Tuberculosis Experimental. VII. Hidracidas y Derivados de Acidos Heterocíclicos

Revélase la actividad antituberculosa de varias hidracidas del ácido carboxílico heterocíclico. En esta serie, las más activas son la hidracida del ácido 2-furoico, la hidracida del ácido carboxílico-2-tiofeno y la hidracida del ácido carboxílico-5-imidazol. Ninguno de los compuestos ensayados es tan activo como la isoniacida.

RESUME

Chimiothérapie et tuberculose expérimentale. VII. Hydrazides d'acide hétérocyclique et leurs dérivés

Le pouvoir antituberculeux de plusieurs hydrazides d'acide carboxylique hétérocyclique est révélé. Les plus actives de cette série sont l'hydrazide de l'acide 2-furfurane-carbonique, l'hydrazide de l'acide thiophéno-2-carboxylique et l'hydrazide de l'acide imidazole-5-carboxylique. Aucun des composés expérimentés ne s'est montré aussi actif que l'isoniazide.

REFERENCES

(1) Bernstein, J., Jambor, W. P., Lott, W. A., Pansy, F., Steinberg, B. A., and Yale, H. L.: Chemotherapy of experimental tuberculosis: VI. Derivatives of isoniazid, Am. Rev. Tuberc., 1953, *67*, 354.

(2) Yale, H. L., Losee, K., Martins, J., Holsing, M., Perry, F. M., and Bernstein, J.: Chemotherapy of experimental tuberculosis: VIII. The synthesis of acid hydrazides, their derivatives and related compounds, J. Am. Chem. Soc. (in press).

(3) Rake, G., Jambor, W., McKee, C. M., Pansy, F., Wiselogle, F. Y., and Donovick, R.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: III. The standardized test, Am. Rev. Tuberc., 1949, *60*, 121.

Case Reports

HISTOPLASMOSIS[1]

Report of Two Cases

ROBERT CHARR

(Received for publication July 28, 1952)

Until January, 1950, 138 authenticated cases of clinical histoplasmosis had been reported (1). Between January, 1950, and August, 1952, approximately 40 additional cases were reported, making a total of approximately 178 cases. In approximately one-half of the cases, the lungs were involved. The ages of the patients ranged from a few weeks to the eighth decade of life. Almost 20 per cent of the patients were less than one year of age. Male patients outnumbered the female patients by two to one. These patients lived in twenty-five different states of the United States and in seventeen foreign countries.

CASE REPORTS

The present communication reports 2 patients with clinical histoplasmosis.

Case 1. The patient was a 48-year-old Greek merchant who had come to the United States in 1924 and had lived in Philadelphia practically all of the time. In 1942, he developed a small tender area in the left side of his palate. This area was incised. It drained for a few days and then healed. Shortly thereafter he had an attack of what he considered to be an acute chest cold. His temperature was 101°F.; the fever gradually subsided. In December, 1942, he developed a sore throat with mild fever. On physical examination, the patient was seen to be a well-nourished white male with normal findings in eyes, ears, and nose. The throat appeared reddened. The neck was normal; no palpable lymph nodes were found. The thyroid was normal in size. The chest was essentially normal except for scattered musical rales throughout both lungs. Examinations of the heart and abdomen were negative. The liver and spleen were not enlarged. The extremities were normal. The blood count, urinalysis, and blood Wassermann reaction revealed no abnormalities. Roentgenographic examination revealed a nodule suggestive of a healed primary lesion at the level of the third intercostal space on the right side near the periphery of the lung. About the upper mediastinum on either side of the trachea were increased densities suggesting enlarged lymph nodes, particularly on the right side (figure 1). The hilar shadows were also exaggerated.

Between January, 1943, and October, 1945, the patient returned at least once every six weeks complaining of return of "chest cold" with cough and white mucoid expectoration. The only physical findings of any moment were the scattered musical rales heard most persistently over the right apex. For these "colds," the only medication given was cough mixture.

On October 25, 1945, the patient returned with an extensive herpes zoster involving the right lower intercostal nerves. The lesions persisted for more than a month and then gradually cleared. X-ray therapy was given over the involved nerves with considerable benefit.

Between May, 1946, and February, 1949, the patient returned a number of times with

[1] From the Jefferson Medical College, Philadelphia, Pennsylvania; and the Philadelphia Tuberculosis and Health Association, Philadelphia, Pennsylvania.

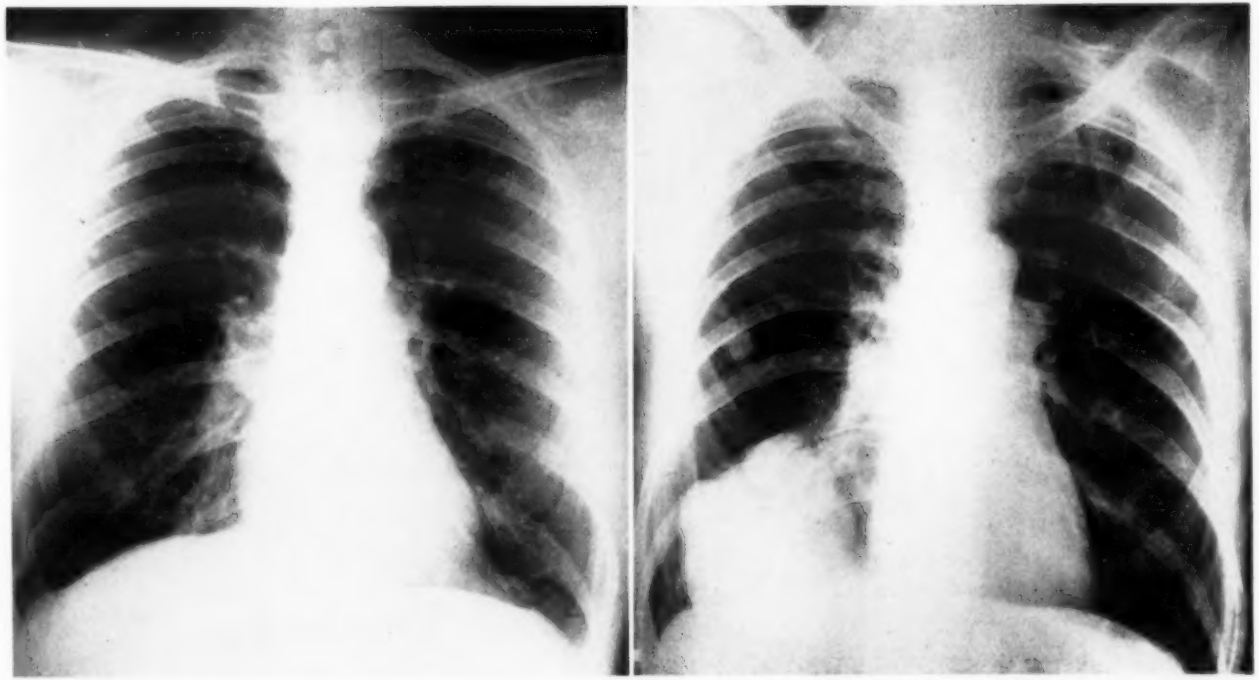


FIG. 1. (Left) Roentgenogram taken in December, 1952, showing widened upper mediastinum, particularly on the right side.
FIG. 2. (Right) Roentgenogram taken in December, 1952, showing massive consolidation in the right lower lobe. (Courtesy of Dr. Paul C. Swenson)

the same complaint of cough. Repeated roentgenographic examinations of the chest showed nothing unusual other than the increased density on either side of the trachea, particularly on the right.

On February 25, 1949, the patient returned with hoarseness, cough, and mucoid expectoration. The larynx was normal. The chest contained musical rales which were more numerous over the right apex. On March 1, 1949, he developed a sore throat. No ulcerations or swelling of the tissues of the pharynx were noted. At this time, as in February, 1942, the patient complained of a painful swelling in the left side of the soft palate. It was thought the swelling of the palate was due to ill-fitting dentures. The swelling persisted and was finally incised by Dr. Wagers. The incision permitted a small amount of serosanguineous fluid to exude for a few days. Following the incision, the lesion gradually became larger despite treatments.

In September, 1949, he entered the Jefferson Hospital and remained there continuously for two and one-half years until his death in February, 1952.

The patient reacted to the histoplasmin skin test on two occasions. The tuberculin test, Wassermann reaction, and the muscle biopsy for periarteritis nodosa were negative. Throughout the entire illness, cough and mucoid expectoration continued. In November, 1951, the cough became more pronounced with blood-tinged sputum. The sputum was examined for tubercle bacilli but none was found. At this time the chest roentgenogram showed an area of density in the right lower chest with a smaller nodule in the right midlung field (figure 2). The bronchoscopic examination was essentially negative. The study of the bronchial secretion for tumor cells was negative. By now the entire soft and hard palates eroded away. A gastrostomy was done, and some degree of nutrition was maintained through the gastrostomy opening.

Therapy consisted of penicillin, aureomycin, chloramphenicol, streptomycin, bacillomycin, stilbamidine, arsphenamine, sulfadiazine, Promin, potassium iodide, nitrogen mustard, and deep radiation, all of which were tried in adequate doses for a sufficient length of time. None of these therapies was of definite value. The ulcer in the palate grew larger and eventually opened into the left maxillary sinus. The mass in the right lower lobe grew larger with an increase in cough and expectoration. On February 22, 1952, the patient died of massive pulmonary hemorrhage.

Studies of the granulomatous tissues removed by biopsy of the ulcer in the soft palate showed the characteristic bodies of *Histoplasma capsulatum* within the cytoplasm of the reticulo-endothelial cells.

Post-Mortem Findings

The post-mortem examination made by Dr. Breckenridge showed an extensive necrosis of the left maxillary region. The left nares opened into the oral cavity. There was ulceration of the soft and hard palates, particularly on the left side.

Each pleural cavity contained approximately 50 cc. of yellowish fluid. Approximately 25 cc. of the same type of fluid were present in the pericardial sac. There were dense adhesions between the dome of the diaphragm on the right side and the under surface of the right lower lobe.

The heart weighed 350 gm. The findings were normal except for a small pinkish-gray vegetation on the posterior cusp of the aortic valve.

The right lung weighed 800 gm. On either side of the trachea and the bronchi were numerous large lymph nodes. None of them showed gross evidence of necrosis or caseation. Surrounding the right lower bronchus was a large firm mass measuring approximately 6 cm. in diameter. In the center of this mass were several abscesses. The right upper and middle lobes and the left lung were essentially normal except for congestion. The tracheobronchial tree appeared congested. On the false vocal cords were two small scars.

The spleen weighed 190 gm.; and the liver, 2,250 gm. Neither organ showed anything unusual. The adrenals, gallbladder, pancreas, thyroid gland, kidneys, blood vessels, bone marrow, gastrointestinal tract, and the brain were essentially normal.

The histologic examination of the tissues from the granulomatous lesions in the nasopharynx and the right lung showed the typical intracellular bodies of *Histoplasma capsulatum*.

The tender swellings in the left side of the soft palate which first appeared in 1942 and reappeared in 1949 may be considered as related and were presumably both brought about by histoplasmosis. It is generally recognized that most of the cases of clinical histoplasmosis first develop somewhere in the mouth or larynx. It is conceivable that an ill-fitting denture may cause a local area of tissue destruction favorable to the growth of histoplasma. In a case of systemic histoplasmosis, it has been reported that the initial focus occurred in an area of dental caries (2).

Cough, expectoration of white mucoid sputum, and persistent musical rales throughout the chest and most often over the right apex were the persistent findings in this patient throughout his entire illness for ten years. Numerous roentgenographic examinations of the chest between 1942 and 1952 showed nothing unusual other than the increased shadows on either side of the trachea. Not until approximately three months prior to his death did he develop massive infiltration in the right lower lobe. This suggests that possibly the lymph nodes which were found to be numerous about the trachea at necropsy harbored the organisms throughout the years.

Unfortunately, the vegetation on the aortic valve was not examined histologically. The vegetation could have been due to histoplasma. Endocarditis due to *Histoplasma capsulatum* has been reported (3). Ten cases have been reported; in 6 the pathologic lesions were those of an infective endocarditis, in 2 the organisms were present only in the myocardium.

Case 2. The patient was a 62-year-old house painter who was admitted to the Jefferson Hospital on November 3, 1950, complaining of difficulty in swallowing, hoarseness, cough, and wheeze in the chest of eight weeks' duration. The past history was irrelevant.

The present illness began in August, 1950, when the patient noted hoarseness together with painful and difficult swallowing of liquids as well as of solid foods. He had lost approximately fifteen pounds in weight since September, 1950. At the same time he developed a cough with expectoration of mucoid sputum and a wheeze throughout his chest. At no time was there blood in the sputum. The patient consulted his family physician and, on laryngoscopic examination, a growth on the right vocal cord was discovered.

At the time of admission to Jefferson Hospital, the physical examination showed the right pupil was fixed; the left pupil reacted slowly to light. Ophthalmoscopic examination revealed no abnormalities. Ears, nose, mouth, and throat were normal. In the larynx there was a granular elevation in the right arytenoid which extended slightly into the interarytenoid space. This elevation did not involve the aryepiglottic fold. A slight limitation of motility of the right arytenoid and some accumulation of secretion in the right pyriform sinus were noted. The left vocal cord appeared normal. No nodes were palpable in the neck. The trachea was in the mid line.

The chest appeared thin and flat; the respiratory expansion was limited on both sides. The breath sounds were distant throughout, and no rales were heard.

The heart, abdomen, external genitalia, skin, and extremities were normal.

Rectal examination showed polyps on the right side of the rectum. The fecal material was mixed with fresh blood.

Roentgenographic examination of the chest (figure 3) on October 27, 1950, showed numerous deposits in both apices with considerable fibrosis. From the roentgenographic appearance, it was difficult to determine whether the changes were tuberculous in origin or due to some other infection. The heart and diaphragm, as well as the ribs, appeared normal. On October 28, 1950, direct laryngoscopy was performed and revealed a lesion on the posterior and inner aspect of the right arytenoid. The lesion appeared to be superficially

ulcerated. Biopsy was done and secretions were obtained for cancer cell study. The secretions obtained from the larynx showed no acid-fast organisms.

On October 28, 1950, the tissue removed from the larynx showed the characteristic bodies of *Histoplasma capsulatum* within the cytoplasm of the cells in granulomatous tissue. On November 3, 1950, the histoplasmin skin test was negative.

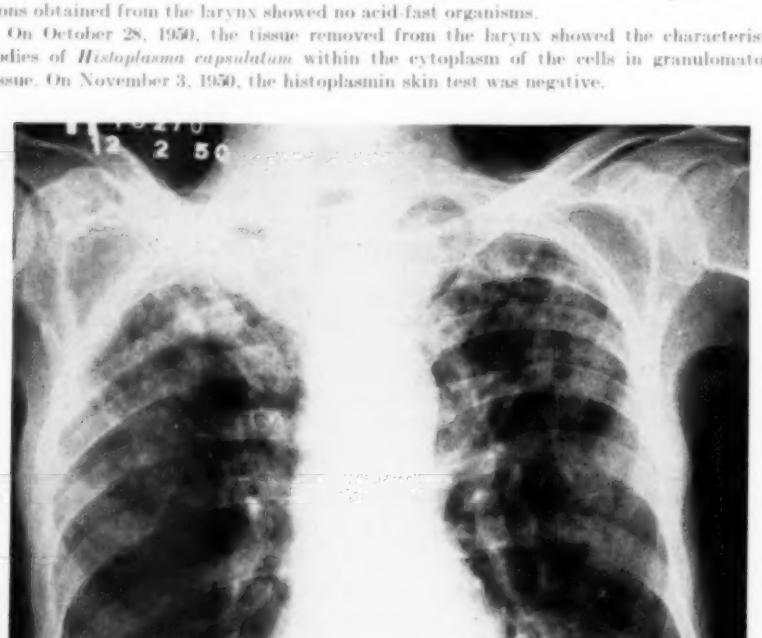


FIG. 3. Roentgenogram showing diffuse infiltration in the upper half of each lung with cavitation in the left upper lobe. (Courtesy of Dr. Paul C. Swenson)

The sternal marrow was aspirated on November 13, 1950, and was examined for histoplasma, but none was found. A report from the Army Medical Department, Washington D. C., on November 24, 1950, stated that the histoplasmin complement-fixation reaction of the patient's serum was negative. The cutaneous reaction to tuberculin was negative.

In the latter part of December, 1950, the patient developed considerable shortness of breath, even while resting, and pronounced difficulty in swallowing. He also developed an ulceration on his tongue which measured approximately 4 mm. in diameter.

Roentgenographic examination of the hands and feet on November 6, 1950, revealed no

abnormalities. The barium enema made on November 7, 1950, showed only polyps of the colon. A roentgenographic skeletal survey on November 30, 1950, showed no changes in the pelvis, spine, legs, and arms. On December 2, 1950, roentgenographic examination of the chest showed a beginning excavation in the right apex which appeared to be tuberculous in origin. Gastroscopic examination on November 30 and bronchoscopic examination on December 30 revealed no abnormalities, but acid-fast organisms were present in the bronchial secretions.

On November 16, 1950, a small piece of tissue was removed from the posterior portion of the tongue and revealed changes typical of histoplasmosis on histologic examination. A small piece of tissue was removed from the vestibule of the nose on November 8, 1950, and also revealed histoplasma on histologic examination. A rectal polyp was removed on November 14, 1950, and it also showed histoplasma. Liver biopsy was made on November 22 and it too showed histoplasma. From these tissues, typical *Histoplasma capsulatum* was easily isolated by culture.

The patient's clinical course was progressively downhill with increasing dysphagia, weight loss, and weakness. His cough became worse with an increase in the amount of expectoration. He received streptomycin, 1 gm. daily, which had no effect. He presented the manifestation of Addison's disease and it was thought that this might be due to possible involvement of the adrenal glands. Consequently, he received 2 mg. of desoxycorticosterone acetate intramuscularly every day and the salt intake was increased. This treatment had no effect, and on February 15, 1951, the patient died.

Post-mortem examination on February 16, 1951, performed by Dr. McCloskey, revealed:

Histoplasmosis of the larynx, both lungs, both adrenals, small intestine, large intestine, and possibly of the spleen, liver, and cervical and para-aortic lymph nodes
Pulmonary tuberculosis and latent syphilis (clinical)
Severe atherosclerosis of the aorta
Severe emaciation
Ulceration of the nares
Decubitus ulcer
Acute arthritis of right knee
Brown atrophy of the heart
Thickening of the tricuspid valve

Comment: In this patient it is difficult to determine whether the histoplasmosis followed or preceded the tuberculosis. It is of interest that, despite the extensive dissemination of histoplasmosis, both the skin test and complement-fixation test were negative. In this respect, the failure of the patient to react to the skin test and the negative complement-fixation reaction are analogous to the phase of anergy observed in certain cases of disseminated tuberculosis.

DISCUSSION

The close resemblance of histoplasmosis to diseases such as tuberculosis, sarcoidosis, pneumonoconiosis, infectious mononucleosis, viral pneumonia, Addison's disease, kala-azar, lymphoma, and carcinomatosis bring about confusion in diagnosis. The diagnosis of histoplasmosis can be made during life, but in approximately one-half of the reported cases it could not be made until the post-mortem studies were completed. The causative microorganism is difficult to identify in the tissues even when they are examined with this disease in mind. The histoplasma bodies are minute and often so widely scattered in the tissues that prolonged search under the highest magnification by an expert is necessary (4).

Unless the organisms are numerous in the tissues, they are difficult to culture. In ap-

proximately only one-half of the proved cases has it been possible to isolate the organisms. Likewise, animal inoculation has been positive for histoplasma in approximately 50 per cent of the cases in which the diagnosis was proved by autopsy.

The histoplasmin skin test is helpful in mass surveys for case finding, but it may give negative or equivocal results in known cases. The complement-fixation test is considered more reliable but even this may give equivocal reaction in a known case. In Case 2, both the skin and complement-fixation tests were negative although it was possible to isolate histoplasma from the tissues removed for biopsy studies. The skin, complement-fixation, and precipitin reactions were positive in approximately one-half of the reported cases. The reliability of these tests is doubtful in certain cases because of possible cross-reaction with antibody formed in response to infection with other fungi.

Histoplasma may invade any tissue or organs. The most frequently involved organs in the order of frequency are the lymph nodes, liver, lungs, spleen, adrenal glands, intestines, bone marrow, kidneys, and oropharynx (1). In the lungs the lesions are usually granulomatous tissue containing exudation, consolidation, fibrosis, and at times necrosis with liquefaction. The exudate is composed of polymorphonuclear cells and lymphocytes. The proliferation and fibrosis consist of macrophages and fibroblasts. The macrophages contain the histoplasma in their cytoplasm. The lesions may be single or multiple. Any portion of the lung may be involved. The regional lymph nodes may or may not be involved. When the involvement is superficial, the visceral pleura becomes affected with exudation and fibrosis. In such cases the pleural fluid may contain histoplasma. Superficially located lesions may ulcerate but such an ulcer has no diagnostic characteristics. Consequently, it is difficult to differentiate it from tuberculosis, syphilis, or neoplastic disease. This is particularly true when the ulcer is on the larynx, palate, lips, or tongue. Superficial ulceration in histoplasmosis is known to have occurred on the face, neck, hands, buccal mucosa, alveolar ridge, pharynx, esophagus, anus, and external genitalia.

In the reported cases, when the lungs were involved the most common symptom seemed to be frequent "colds." The sputum is usually mucoid and occasionally blood tinged. At times the severity of cough increases with sensation of tightness in the chest, wheeze, and fever. The patients usually considered these symptoms to represent an acute cold. Some patients had such symptoms more or less continuously for several years.

Roentgenograms of the chest may be entirely normal in some patients despite chronic cough and mucoid expectoration. There is no specific roentgenographic appearance for pulmonary histoplasmosis. In some there is exaggeration of the peribronchial tissue markings extending outward from the hilar regions, and in others there are areas of infiltration or consolidation. In some there are scattered nodules, either discrete or conglomerate, in one or both lungs. In some the findings closely resemble chronic pulmonary tuberculosis with cavitation, and in others the findings cannot be differentiated from the classical roentgenographic appearance of sarcoidosis. Nodular calcification of the lungs was found occasionally in active histoplasmosis.

Histoplasma capsulatum has been found in dogs and rodents. It also has been found in the intestinal tract of persons without clinical histoplasmosis (6). The organism has been found in the soil and the rivers running through such a soil (7). The question whether histoplasmosis is transmissible from man to man is still unanswered. It has been postulated that a person may be infected by his dog, which in turn is infected by a rodent which has received the organism from the soil.

The longest survival after the establishment of the diagnosis of clinical histoplasmosis has been approximately two years. When the infection is mild, the defense mechanism of the body seems to hold the disease in abeyance. But when the infection is outspread, pre-

sumably in a person with low resistance, the condition appears hopeless. None of the antimicrobial drugs available today seems to have specific therapeutic value in histoplasmosis. Ethyl vanillate has been tried with some success (8).

SUMMARY

Two cases of histoplasmosis have been reported in both of which the correct diagnosis was made before death. In one, the tissues removed from the ulcer in the soft palate showed the characteristic histoplasma bodies in the cytoplasm of the macrophages. In the other, the histologic studies as well as the culture of the tissues and exudate confirmed the diagnosis of histoplasmosis. When the diagnosis of histoplasmosis is suspected, reliance cannot be placed on skin or complement-fixation tests. The diagnosis can be made tentatively by various clinical and pathologic features and definitely by the identification of the organisms.

There is no specific therapy for histoplasmosis, and the disease is probably more prevalent than the reports indicate.

SUMARIO

Histoplasmosis

Descríbense 2 casos de histoplasmosis clínica. En ambos se hizo el diagnóstico acertado antes de la muerte. En uno, los tejidos extirpados de la úlcera del paladar blando revelaron los típicos cuerpos del histoplasma en el citoplasma de los macrófagos. En el otro, los estudios histológicos, así como el cultivo de los tejidos y del exudado, confirmaron el diagnóstico de histoplasmosis. Cuando se sospecha este diagnóstico, no hay que atenerse a la cutirreacción y/o a la prueba de fijación del complemento. Cabe hacer el diagnóstico tentativamente por las características clínicas y anatomopatológicas y definitivamente por la identificación de los microbios.

En la histoplasmosis, no hay terapéutica específica. Claro está que mucho depende de la gravedad de la infección y de la capacidad del enfermo para combatirla. La histoplasmosis es probablemente más frecuente de lo que indican las comunicaciones publicadas.

RESUME

Histoplasmose

Description de deux cas d'histoplasmose clinique. Dans ces deux cas, le diagnostic avait été posé avant le décès. Dans un des cas, les tissus prélevés sur l'ulcération du voile du palais montraient les corpuscules histoplasmiques caractéristiques dans le cytoplasme des macrophages. Dans l'autre, les examens histologiques et la culture des tissus et de l'exudat ont confirmé le diagnostic d'histoplasmose. Quand le diagnostic d'histoplasmose est envisagé, il ne faut pas se fier au test cutané ou au test de fixation du complément, ou à ces deux tests. Divers caractères cliniques ou histo-pathologiques permettent un diagnostic provisoire qui est confirmé par l'identification du champignon.

Il n'y a pas de traitement spécifique de l'histoplasmose. Il est évident que la sévérité de l'infection et les moyens de défense du malade pour lutter contre l'affection sont des facteurs importants. L'histoplasmose est probablement plus fréquente que les rapports ne l'indiquent.

Acknowledgment

The author is indebted to Dr. Hobart A. Reimann for his permission to report these cases.

REFERENCES

(1) Hodgson, C. H., Weed, L. A., and Clagett, O. T.: Pulmonary histoplasmosis, J.A.M.A., 1951, *145*, 807.

(2) Snoke, P.: Histoplasmosis, Pennsylvania M. J., 1949, *54*, 957.
(3) Fawell, W. N., Browns, H. L., and Ernestene, A. C.: Vegetative endocarditis due to *Histoplasma capsulatum*, Cleveland Clinic Quarterly, 1951, *18*: 305.
(4) Reimann, H. A., and Price, A. H.: Histoplasmosis in Pennsylvania, Pennsylvania M. J., 1949, *52*, 367.
(5) Emmons, C. W., and Ashburn, L. L.: Histoplasmosis in wild rats, Pub. Health Rep., 1948, *63*, 1416.
(6) Raftery, A.: Subclinical histoplasmosis—gastrointestinal histoplasmosis of children, J.A.M.A., 1951, *145*: 216.
(7) Zeidberg, L. D., Ajello, L., Dillon, A., and Runyon, L. C.: Isolation of *Histoplasma capsulatum* from soil, Am. J. Pub. Health, 1952, *42*, 930.
(8) Christie, A., Middleton, J. G., Peterson, C., and McVickar, D.: Treatment of disseminated histoplasmosis with ethyl vanillate, Pediatrics, 1951, *7*, 7.

PELIOSIS HEPATIS[1]

Report of Two Cases

J. M. YOUNG

(Received for publication August 28, 1952)

Peliosis hepatis is a pathologic condition in which many small dilated cystic, blood-filled spaces are found in the liver. In 1950, Zak (1) reviewed the literature concerning peliosis and reported that he had found the process in a patient who died of carcinoma of the kidney and a lung abscess. Zak's article on the subject was the first to appear in the English literature. Thirty-four of the reported cases of peliosis hepatis are accepted by him as being well authenticated. Of the thirty-one patients in this group in which the cause of death was listed, twenty-seven died of tuberculosis. Other causes of death were carcinoma of the stomach, carcinoma of the rectum, and urinary tract infection. Recently, Zak (2) has heard of two additional deaths from tuberculosis, both of which revealed peliosis. Rosenfeld (3), reporting on pseudomyxoma peritonei, illustrated and described the lesions of peliosis in one of his cases. Recently, Burger and Marcuse (4) made a report on a patient who died of carcinoma of the colon and showed peliosis hepatis. The present report concerns 2 patients who died from advanced pulmonary tuberculosis and showed the lesions of peliosis hepatis on post-mortem examination.

CASE REPORTS

Case 1. A. J., a 63-year-old white male farmer, developed pulmonary tuberculosis in 1929. The disease responded to treatment but recurred in 1946. Following therapy the patient became asymptomatic until 1948, when he developed fever, a productive cough, and weight loss. He entered Kennedy Veterans Administration Hospital for the first time in July, 1948. His sputum became negative for acid fast bacilli during the course of a year of bed rest and supportive therapy for his tuberculosis. In October, 1949, the patient developed symptoms of prostatic obstruction and underwent suprapubic prostatectomy. At this time, smears of sputum were negative, but cultures were positive for tubercle bacilli. The significant findings on physical examination were limited to the chest where post-tussive rales, bronchial breathing, and some dullness to percussion over the upper lung fields posteriorly were noted. A roentgenogram of the chest revealed bilateral infiltrations in both upper lung fields and a cavity 2 cm. in diameter in the right apex. Laboratory examinations disclosed a positive serologic test for syphilis and a corrected erythrocyte sedimentation rate of 44 mm. per hour.

From January until July, 1950, the patient was treated for his tuberculosis with bed rest and supportive therapy, and he remained afebrile. Nevertheless, he developed a second cavity, located in the left upper lobe, and his sputum remained positive for tubercle bacilli.

From July, 1950, until August, 1951, the patient received repeated courses of streptomycin with and without para-aminosalicylic acid (PAS). He showed no significant change in his pulmonary disease, and his weight dropped from 145 to 108 pounds. From August until December, 1951, he received Promin, but his disease steadily progressed. Roentgenograms revealed a marked increase in the pulmonary infiltrations bilaterally and development of a huge cavity in the lower left lung field. His temperature, which had previously been normal, began rising to 104°F. in November, 1951, and he died on December 26, 1951.

[1] From Laboratory Service, Veterans Administration Medical Teaching Group, Kennedy Hospital, Memphis 15, Tennessee.

Post-Mortem Findings

The body weighed approximately 100 pounds and showed severe emaciation. Both pleural cavities were obliterated by dense adhesions and the visceral pleura measured 3 to 4 mm. in thickness. There was a cavity 12 cm. in diameter in the left lower lobe and the lateral wall was formed by thickened pleura. The cavity was partly filled with 200 cc. of yellow, purulent fluid in which there were bits of necrotic tissue. The left lung weighed 860 gm. and was firm and grayish-pink in color. Section revealed extensive honeycombing throughout and no crepitant tissue. There was bronchiectatic dilatation of the smaller bronchi, and their lining was reddened and granular. The right lung weighed 800 gm. and the cut surface disclosed a cavity 5 cm. in diameter in the upper lobe, smaller areas of caseation necrosis in the upper and middle lobes, and reddening of the bronchial lining. All of the bronchi contained grayish-green mucopurulent material.

The spleen, adrenals, kidneys, and gastrointestinal tract showed chronic passive hyperemic changes. The liver weighed 1,460 gm. and was deep brown in color. Beneath the capsule, numerous small reddish-purple spots were seen. The cut surface also disclosed many of these small cystic blood-filled spaces measuring as much as 3 mm. in diameter (figure 1). Prominent chronic passive hyperemia was present.

Sections from the lungs revealed extensive scarring and tubercle formation with superimposed tuberculous pneumonia and widespread caseation necrosis. Functional pulmonary tissue was greatly reduced.

Examination of sections of the liver showed very marked congestive changes, most prominent in the pericentral region. The sinusoids were distended with erythrocytes and occasionally formed small lakelike spaces (figure 2). There were many focal areas with polymorphonuclear cell infiltration, and in these places the liver cells were frequently undergoing degenerative changes (figure 3). The actual formation of the peliotic focus seemed to follow disintegration and removal of these liver cells (figure 4). Throughout the sections there were many of the peliotic spaces which were filled with erythrocytes, fibrinous material, leukocytes, and liver cell debris. The leukocytes and erythrocytes often showed separation, perhaps due to settling (figure 5). The peliotic foci did not contain clotted blood. The surrounding liver cell cords were compressed, and the surrounding sinusoids often contained many acute inflammatory cells. The peliotic foci were usually near the central vein and occasionally communicated with it widely. Reticulum stains showed these spaces lined only partly by endothelium. Often these foci occurred in groups with little intervening liver tissue. Many of the liver cells and Kupffer cells contained iron and bile pigment granules. Stains of sections for tubercle bacilli were negative.

Throughout the sections of the spleen, the red pulp was very congested. Scattered lakelike, blood-filled spaces were observed although these were smaller than the ones in the liver. They occasionally communicated directly with the larger veins and occurred in the regions where congestion was most prominent.

Case 2. J. B. was a 60-year-old Negro male who developed night sweats and weight loss in 1949 and was diagnosed as having far advanced bilateral pulmonary tuberculosis and tuberculous bronchitis. He had several admissions to the hospital and often left against medical advice. His infection did not respond to streptomycin and the other usual therapeutic measures.

Roentgenographic examination revealed extensive cavitation in the right upper lobe and scattered lesions throughout the remainder of the right and left lungs. Examination of the sputum was repeatedly positive for tubercle bacilli.

During the last hospitalization, the patient's course was rapidly downhill, and he died on June 20, 1952.

FIG. 1. *Case 1.* Liver showing prominent congestion and numerous small blood-filled peliotic foci.

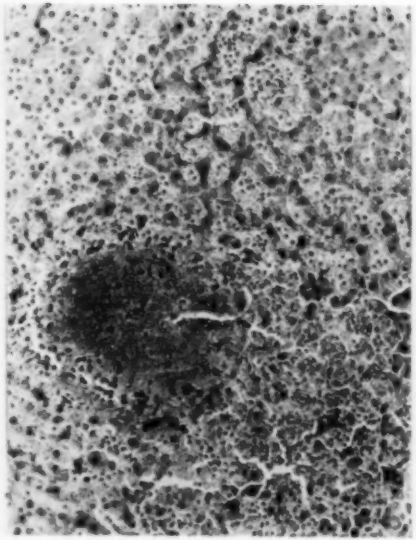


FIG. 2. *Case 1.* Liver showing broad sinusoids and one small peliotic focus filled with fibrin. (H and E × 100)

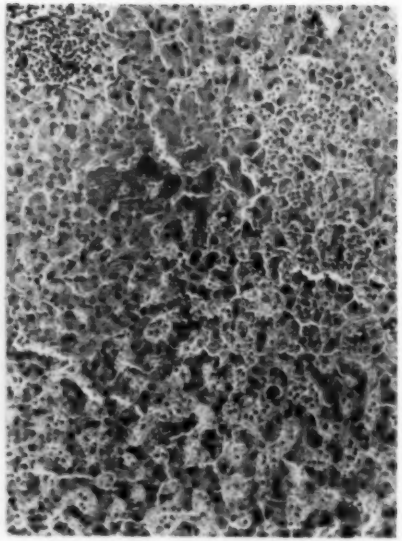


FIG. 3. *Case 1.* Liver. Note polymorphonuclear cells in sinusoids and degenerating liver cells. (H and E × 100)

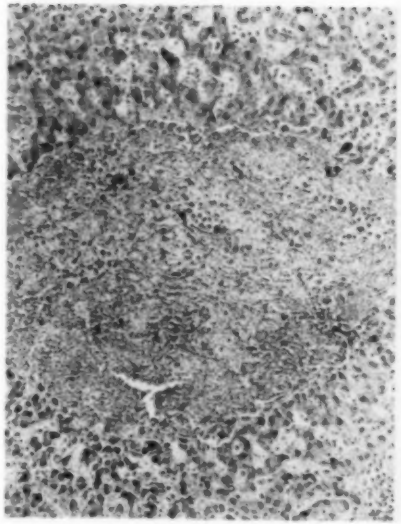


FIG. 4. *Case 1.* Liver. Larger peliotic focus filled with cellular debris and fibrinous material. (H and E × 100)

FIG. 5. *Case 1.* Liver. Cystic space showing layering of cells and fibrinous material and compression of surrounding liver cell cords. (H and E × 60)

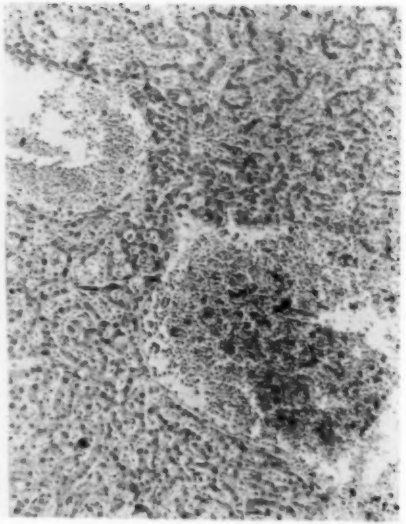


FIG. 6. *Case 2.* Liver. Peliotic foci. Note absence of inflammatory reaction and necrosis.

Post-Mortem Findings

The body was very emaciated and weighed only 95 pounds. Both pleural cavities were obliterated by dense adhesions. When the right pleura was stripped away from the chest wall, a large multiloculated cavity was entered in the right upper lobe. This measured 15 cm. in diameter and was partly filled with thick grayish-green purulent material. The left lung weighed 750 gm. and the cut surface disclosed many foci of tuberculous pneumonia and a cavity in the lower lobe. The right lung, in addition to the large upper lobe cavity, contained many foci of pneumonitis. The bronchi of both lungs were partly filled with greenish mucoid material. The heart disclosed moderate right ventricular hypertrophy and weighed 320 gm. The right ventricular wall measured 0.7 cm. in thickness.

The liver and gallbladder weighed 1,450 gm. The capsular and cut surface of the liver disclosed marked passive congestion. Near the capsular surface of the dome of the right lobe there were numerous dilated blood-filled spaces measuring up to 0.2 cm. in diameter. None of these was deeper than 2.0 cm.

Microscopic examination of the tissues confirmed the gross findings. The liver sections disclosed broad blood-filled sinusoids and many peliotic foci similar to those in *Case 1* (figure 6). There was almost no necrosis or inflammatory reaction present. Again, most of these foci were near the central vein region. No definite lining was present in these spaces. Stains of liver sections for tubercle bacilli were negative.

DISCUSSION

Many theories have been advanced to explain the formation of the lesions in peliosis hepatis. These include (*a*) congenital malformation exaggerated by congestion, (*b*) varicosities, (*c*) ruptured vessels with or without preceding inflammation, and (*d*) focal hepatic necrosis followed by hemorrhage.

Senf (5) was able to reconcile many of the conflicting views. He believes that the initial lesion is a focus of necrosis of liver cells and that these are miliary throughout the liver. Next, by sudden increase of pressure in the hepatic veins (by coughing, et cetera), hemorrhage into the necrotic focus occurs, followed by resorption of the necrotic debris and formation of a fibrous wall about the cystic space.

Observations on the present cases support Senf's views to a certain extent. In *Case 1*, there were many foci where acute inflammation and necrosis occurred, and these seemed to precede formation of the peliotic focus. Nevertheless, no fibrous wall was seen about the peliotic foci in this case or in Zak's case. Whatever the exact pathogenesis, certainly marked congestion, focal inflammation and necrosis, and advanced pulmonary disease are frequently important factors. The lesions in *Case 1* are believed to be the early ones. In *Case 2*, however, the peliotic foci were better defined and represented a later stage of the process.

These two cases occurred in a small series of deaths from tuberculosis, which suggests that peliosis hepatis is more common than is indicated in the literature.

SUMMARY

Two cases of fatal pulmonary tuberculosis are reported in which peliosis hepatis was found on post-mortem examination.

SUMARIO

Peliosis Hepática: Presentación de Dos Casos

Dos casos letales de tuberculosis pulmonar que mostraban peliosis hepática son presentados y discutidos aquí. En ambos, había tuberculosis pulmonar muy avanzada y adherencias pleurales.

RESUME

Péliose hépatique: Rapport sur deux cas

Deux cas mortels de tuberculose pulmonaire avec péliose hépatique sont rapportés et étudiés. Dans les deux cas il s'agissait d'une tuberculose pulmonaire à un stade avancé avec adhérences pleurales.

Addendum

Since submitting this report, the writer has examined at autopsy another patient, a 30-year-old Negro male, who died with far advanced pulmonary tuberculosis and showing the lesions of peliosis hepatis. The lesions were similar to those described in *Case 2* and were located in the right lobe of the liver near the diaphragmatic surface.

These three cases occurred in autopsies on fifteen patients who died with advanced pulmonary tuberculosis. This indicates that the lesions of peliosis hepatis are certainly not rare, as has been the opinion heretofore.

REFERENCES

(1) Zak, F. G.: Peliosis hepatis, Am. J. Path., 1950, *26*, 1.
(2) Zak, F. G.: Personal communication.
(3) Rosenfeld, E. D.: Peritoneal pseudomyxoma, Arch. Path., 1949, *48*, 255.
(4) Burger, R. A., and Marcuse, P. M.: Peliosis hepatis: Report of case, Am. J. Clin. Path., 1952, *22*, 569.
(5) Senf, H. W.: Über die Entstehung der verschiedenen Formen miliarer Leberblutungen (peliosis hepatis), Virchow's Arch. f. Path. Anat., 1926, *259*, 366.

Notes

LACK OF SIGNIFICANT IN VITRO SENSITIVITY OF MYCOBACTERIUM TUBERCULOSIS TO PYRAZINAMIDE ON THREE DIFFERENT SOLID MEDIA

Since the introduction of pyrazinamide for the treatment of tuberculosis, efforts have been made to develop a satisfactory method for studying the *in vitro* activity of this agent. Dessau and his associates[1] recently described a technique using Dubos liquid medium but because of its shortcomings suggested that a solid medium might be better for determining the susceptibility of tubercle bacilli to the drug. In view of this report and because of the present writers' own difficulties with liquid media, it was believed desirable to investigate the value of solid media for this purpose. In the present study, three media were tried, namely, American Trudeau Society,[2] blood,[3, 4] and Herrold[5] media.

The American Trudeau Society (ATS) and Herrold media were modified somewhat and the formulas and methods of preparation were as follows:

ATS medium:

Fresh egg yolk	400.0 ml.
Potato water with 8 per cent glycerine	333.0 ml.
Malachite green (1 per cent aqueous solution)	10.0 ml.

Preparation: The potato water was made by adding 100 gm. of finely ground potato to enough water containing 8 per cent glycerine to give a final volume of 333.0 ml. This mixture was then sterilized by autoclaving at fifteen pounds pressure (121°C.) for twenty minutes, after which it was cooled to 45°C. in a water bath. The egg yolk and malachite green were then added and the ingredients thoroughly mixed. The medium was then dispensed in equal quantities to five Erlenmeyer flasks and the desired concentrations of pyrazinamide[6] were added to four of the flasks but not to the fifth, which was used for the control tubes. Each flask was thoroughly shaken, and approximately 3.5 ml. of medium were then added aseptically to sterile screw-cap tubes, after which it was coagulated and sterilized by inspissation at 90°C. for one and one-half hours.

Herrold medium:

Fresh egg yolk	45.0 ml.
Nutrient agar	3.0 ml.
Glycerine (analytical grade)	4.0 ml.
Water (distilled)	150.0 ml.
Malachite green (2 per cent aqueous solution)	1.3 ml.

Preparation: The medium was prepared in five Erlenmeyer flasks by dissolving the agar in the glycerine water by heating. The mixture was then sterilized by autoclaving at fifteen pounds pressure (121°C.) for fifteen minutes, after which it was cooled to 45°C. in a

[1] Dessau, F. I., Burger, F., Yeager, R. L., and Kulish, M.: Am. Rev. Tubere., 1952, *65*, 635.

[2] American Trudeau Society: Report of the Committee on Evaluation of Laboratory Procedures, Am. Rev. Tubere., 1946, *54*, 428.

[3] Tarshis, M. S., and Frisch, A. W.: Am. J. Clin. Path., 1951, *21*, 101.

[4] Tarshis, M. S., and Frisch, A. W.: Am. J. Clin. Path., 1951, *21*, 720.

[5] Herrold, R. D.: J. Infect. Dis., 1931, *48*, 236.

[6] Samples of the pure powder were kindly supplied for study by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York. Lot Nos. 7 8717 and 7 8886.

water bath. The egg yolk and desired concentrations of pyrazinamide were then added to four of the flasks, and the egg yolk alone to the fifth flask, which was used for the control tubes. Each flask was thoroughly shaken and approximately 3.5 ml. of medium were dispensed aseptically into sterile screw-cap tubes and allowed to harden into slants. The flasks were kept in beakers of water in the bath to prevent tipping when the medium became depleted during the tubing process, and were shaken frequently to maintain thorough mixture.

Blood medium:

Blood agar base (Difco)[7]	3.0 gm.
Glycerine (analytical grade)	1.0 ml.
Human bank blood (containing ACD solution, *see* below)	15.0 ml.
Water (distilled)	84.0 ml.

Preparation: The medium was prepared in five Erlenmeyer flasks by dissolving the blood agar base in the glycerine water by heating. The mixture was then sterilized by autoclaving at fifteen pounds pressure (121°C.) for fifteen minutes, after which it was cooled to 45°C. in a water bath. The blood and desired concentrations of pyrazinamide were then added to four of the flasks, and the blood alone to the fifth flask, which was used for the control tubes. Tubing was carried out as described in the preparation of Herrold medium. Outdated bank-blood three to five weeks old was used and contained ACD solution as follows: citric acid U.S.P., 0.50 gm.; sodium citrate U.S.P., 1.37 gm.; and dextrose U.S.P., 2.45 gm., per each 100 ml. of transfusion solution.[8] All media were tested for sterility and stored in the refrigerator for one to seven days before use.

Approximately 72-hour specimens of sputum from tuberculous patients were concentrated by the trisodium phosphate technique and the sediments cultured on Corper-Cohn and penicillin-blood medium.[9] When growth was adequate, subcultures were made to modified Dubos liquid medium in the following manner. To ensure representative sampling, the inoculating loop was moved carefully through the entire culture, and then three heavy loopfuls of organisms were inoculated into the liquid medium. After approximately six to ten days, depending upon the amount of dispersed growth, the control tubes and those containing the pyrazinamide were then inoculated with 0.1 ml. amounts from each culture, which was checked for purity before use. No attempt was made to standardize the inoculum since variations within a fairly wide range do not significantly influence the end results. After inoculation, all tubes were placed in a nearly horizontal position to permit uniform distribution of the organisms. The cultures were incubated for twenty-eight days at 37° C., after which time readings were made. The final degree of growth was recorded by the method recommended by Steenken,[10] which was as follows:

4+ = surface of medium entirely covered
3+ = growth not quite confluent
2+ = innumerable colonies
1+ = 50 to 200 colonies
Actual colony counts made if less than 50

The patients selected for study had previously received various antimicrobial agents. In the present investigation they were treated with pyrazinamide alone, pyrazinamide in combination with streptomycin or dihydrostreptomycin, or in combination with streptomycin and *p*-aminosalicylic acid. At first all patients received 2.8 gm. of pyrazinamide

[7] Difco Laboratories, Inc., Detroit, Michigan. Control No. 393371.

[8] Approximately 500 ml. of blood were collected in Baxter Transfuse Vac bottles containing 120 ml. of ACD solution. Baxter Laboratories, Inc., Morton Grove, Illinois.

[9] Tarshis, M. S.: J. Lab. & Clin. Med., 1952, *40*, 628.

[10] Steenken, W., Jr.: Personal communication.

daily and after two weeks the dosage was changed to 3.0 gm. daily. Treatment varied from six to sixteen weeks. Organisms were tested for drug susceptibility prior to therapy and at two-week intervals thereafter.

In the initial experiment, susceptibility tests were performed on the blood and ATS media using pyrazinamide in final concentrations of 1, 10, 100, and 1,000 γ per ml. From the data in table 1, it can be seen that no inhibition occurred on the ATS medium, and only partial inhibition on the blood medium containing 100 and 1,000 γ of the drug per

TABLE 1

RESULTS OF PYRAZINAMIDE SUSCEPTIBILITY DETERMINATIONS ON AMERICAN TRUDEAU SOCIETY AND HUMAN BLOOD MEDIA

STRAIN OF M. TUBERCULOSIS	AS	ATS MEDIUM					BLOOD MEDIUM				
		Pyrazinamide γ per ml.					Pyrazinamide γ per ml.				
		None	1	10	100	1,000	None	1	10	100	1,000
1782	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2130	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2213	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	2+
2214	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	2+
2315	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2427	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2460	2	4+	4+	4+	4+	4+	4+	4+	4+	3+	15 colonies
2478	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2535	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2755	P	4+	4+	4+	4+	4+	4+	4+	4+	2+	1+
2757	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	1+
2814	6	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2963	6	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
3014	P	4+	4+	4+	4+	4+	4+	4+	4+	3+	1+
3015	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+
3183	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	1+

Symbols 1+ to 4+ represent final degree of growth after 28 days at 37°C.

AS indicates age of specimen; P, pre-therapy; 2 to 6 indicate period of time in weeks following initiation of therapy.

ml. As indicated previously,[4] this may possibly be due to a greater loss in the potency of the drug in the ATS medium, as a result of inspissation, protein binding, or possibly to other physical factors. Of interest is the fact that no inhibition could be demonstrated on the ATS medium and very little on the blood medium even with those organisms isolated prior to therapy.

In the next experiment, tests were done on ATS, Herrold, and blood media. From the data recorded in table 2, it will be noted that the results were again similar despite the great variation in the composition of the individual media. The findings compared somewhat more closely in this experiment than in the previous one, and again no inhibition could be demonstrated on the ATS medium, and very little on the Herrold and blood media.

The observation that very little *in vitro* activity of pyrazinamide could be demonstrated, even against those organisms isolated prior to the initiation of therapy, are in

agreement with the findings of Cummings[11] and Steenken.[10] Yeager and his associates[12] reported that in untreated patients complete inhibition of growth of tubercle bacilli was seen in Dubos liquid medium containing concentrations of 100 and 1,000 γ of pyrazinamide per ml., and partial inhibition was observed at 10 γ and occasionally at 1 γ per ml. All strains tested after therapy showed growth in a concentration of 100 γ per ml., and organisms from ten such patients grew in 1,000 γ of pyrazinamide per ml. These workers also noted that the drug appeared to show a moderate degree of antituberculous activity in their patients. Walton[13] found that a number of strains were inhibited by 25 to 50 γ of

TABLE 2

RESULTS OF PYRAZINAMIDE SUSCEPTIBILITY DETERMINATIONS ON AMERICAN TRUDEAU SOCIETY, HERROLD, AND BLOOD MEDIA

STRAIN OF M. TUBERCULOSIS	AS	ATS MEDIUM					HERROLD MEDIUM					BLOOD MEDIUM				
		Pyrazinamide γ per ml.					Pyrazinamide γ per ml.					Pyrazinamide γ per ml.				
		None	1	10	100	1,000	None	1	10	100	1,000	None	1	10	100	1,000
5	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
9	2	4+	4+	4+	4+	4+	4+	4+	4+	3+	3+	4+	4+	4+	4+	3+
10	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+
13	8	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
22	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
26	10	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
30	6	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+
31	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
33	2	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+
34	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
36	10	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
37	6	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2898	4	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
2964	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
3185	P	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+

Symbols 3+ to 4+ represent final degree of growth after 28 days at 37°C.

AS indicates age of specimen; P, pre-therapy; 2 to 10 indicate period of time in weeks following initiation of therapy.

the drug per ml. in a liquid medium, but also observed no inhibition of other organisms including pretreatment cultures. He also pointed out that, in general, his experience with pyrazinamide-susceptibility tests was not very satisfactory. Dessau and his associates[1] demonstrated partial inhibition at 1 and 10 γ of pyrazinamide per ml. in Dubos liquid medium, but pointed out that considerable variation in results was encountered from one experiment and from one strain to another. In another study[14] Dessau and co-workers demonstrated that pyrazinamide produced moderate but definite antituberculous ac-

[11] CUMMINGS, M. M.: Personal communication.

[12] YEAGER, R. L., MUNROE, W. G. C., AND DESSAU, F. I.: Am. Rev. Tuberc., 1952, *65*, 523.

[13] WALTON, S. T.: Personal communication.

[14] DESSAU, F. I., YEAGER, R. L., BURGER, F. J., AND WILLIAMS, J. H.: Am. Rev. Tubere., 1952, *65*, 519.

tivity in the guinea pig. Steenken and associates,[10] on the other hand, were not able to demonstrate any *in vivo* activity in the guinea pig.

In summary: In the experiments described, no *in vitro* susceptibility of a number of virulent strains of tubercle bacilli to pyrazinamide could be demonstrated on American Trudeau Society medium, and only partial inhibition was noted on blood and Herrold media containing 100 and 1,000 γ of the drug per ml. These findings were observed even with those organisms isolated prior to the initiation of pyrazinamide therapy.

TUBERCULOSIS RESEARCH SECTION
GENERAL RESEARCH LABORATORY
VETERANS ADMINISTRATION MEDICAL
TEACHING GROUP
KENNEDY HOSPITAL
MEMPHIS 15, TENNESSEE

MAURICE S. TARSHIS
WILLIAM A. WEED, JR.

August 28, 1952

NOTES

PPD INTERNATIONAL STANDARD

The Expert Committee on Biological Standardization of the World Health Organization has established the batch of purified protein derivative (PPD) of tuberculin, prepared by Dr. Florence Seibert of the Henry Phipps Institute, Philadelphia, Pennsylvania, from a human strain of the tubercle bacillus, as the standard for purified protein derivative of mammalian tuberculin.

This decision is a recognition of the long studies carried out at the Henry Phipps Institute by Dr. Seibert under grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

PENNSYLVANIA SOCIETY TO PROMOTE ROUTINE ROENTGENOGRAPHIC SURVEYS IN STATE'S GENERAL HOSPITALS

In line with a resolution passed by the Board of Directors of the Pennsylvania Tuberculosis and Health Society in December, 1951, the Society "will actively promote the routine X-raying by all general hospitals in the state of all hospital and clinic patients admitted and all hospital personnel." An attempt will also be made, in line with a further resolution, to stimulate the operation of similar programs by all mental hospitals in the state.

The main resolution, prepared by the Society's Medical Advisory Committee, is as follows:

Whereas, One of the outstanding problems in tuberculosis control is the discovery of unknown active cases of tuberculosis; and

Whereas, Experience has shown that the general hospital is a most stragegic location in which to conduct a case-finding program; and

Whereas, Hospital X-ray screening finds at least twice as much active tuberculosis as does screening of general population groups; and

Whereas, Case finding by general hospitals is of great benefit to the patient and his family, the hospital staff and personnel, other patients, and the community; and

Whereas, Such a program will aid the hospital in the recruitment of student nurses and personnel, and will reduce workmen's compensation costs; and

Whereas, Relatively few general hospitals in Pennsylvania now X-ray all in-patients, out-patients, and personnel as a routine procedure, be it

RESOLVED, That it is the sense of this meeting that such programs should be adopted by all general hospitals in the State; and be it further

RESOLVED, That the Pennsylvania Tuberculosis and Health Society actively promote the routine X-raying by all general hospitals of all hospital and clinic patients admitted and all hospital personnel.

PUBLIC HEALTH LABORATORY REFRESHER TRAINING COURSES

The following courses are being offered in 1953 at the Communicable Disease Center, Chamblee, Georgia.

Laboratory Diagnosis of Tuberculosis
November 9 to 13, and November 16 to 27
The one-week course is designed for laboratory directors, senior laboratory staff members, physicians, and others of comparable professional standing.
Laboratory Methods in the Study of Pulmonary Mycoses
November 30 to December 18

First consideration is given to personnel from laboratories of official health agencies and other nonprofit organizations.

Application forms can be obtained from the Laboratory Training Services, Communicable Disease Center, P. O. Box 185, Chamblee, Georgia, or from state health officers.

PAN PACIFIC TUBERCULOSIS CONFERENCE TO BE HELD IN MANILA IN 1953

A Pan Pacific Tuberculosis Conference under the joint sponsorship of the Department of Health, Republic of the Philippines, the World Health Organization, and the Philippine Tuberculosis Society will be held in Manila from April 13 to 19, 1953. The opening session will be at the auditorium of the Far Eastern University and the scientific session at the Quezon Institute Conference Hall.

Official invitations through the Department of Foreign Affairs have been issued to 30 countries bordering the Pan Pacific Area. The guest speakers selected by the WHO are Dr. J. Holm, Chairman of the Expert Committee on Tuberculosis of the WHO, who will speak on the social aspect of tuberculosis; and Dr. Eugene Nassau, Bacteriologist of Middlesex County and Harefield Chest Hospital in England, who will deal with the biological aspect of tuberculosis. The National Tuberculosis Association of the U.S.A. will send Dr. Walsh McDermott, Editor of the AMERICAN REVIEW OF TUBERCULOSIS and Associate Professor of Medicine at Cornell University Medical College in New York. He will speak on the clinical aspect of tuberculosis. A guest speaker on medical education in tuberculosis will also be sent by the National Tuberculosis Association. The expenses of the 30 delegates and two guest speakers will be defrayed by the WHO and the two other guest speakers by the National Tuberculosis Association.

Invitations are extended to other health officials of foreign governments and to representatives of various nongovernmental antituberculosis associations and scientific organizations abroad to come as visitors or observers, but at their own expense. They may participate in the discussion during the round table conferences, but are not considered official delegates of the conference.

English will be the official language during the conference. Papers to be read are to be written, preferably in English, and abstracts thereof prepared by the authors themselves. If the papers are written in French or Spanish, abstracts thereof should likewise be prepared by the authors. The reading of the papers will, however, be by invitation only.

Inquiries concerning attendance and participation in the conference may be addressed to the Secretariat: 1739 Rizal Avenue or P.O. Box 2049, Manila, Philippines.

Obituaries

ABRAHAM EZRA JAFFIN

1884–1952

Dr. Abraham Ezra Jaffin, of Jersey City, New Jersey, died on November 25, 1952, at the age of sixty-eight. Dr. Jaffin had been graduated from the College of Physicians and Surgeons at Columbia University, and carried on postgraduate work at the University of Vienna. Dr. Jaffin long cherished an interest in tuberculosis work, was president of the New Jersey Tuberculosis League in 1949, was held in high regard by his fellow physicians, and had been a past president of the Hudson County Medical Society.

In addition to his long and distinguished career in the tuberculosis field, he served loyally many local and state organizations of civic nature, and, at the time of his death, was associated with Mount Sinai Hospital in New York, as well as being clinic chief at the Hudson County Tuberculosis Hospital.

ARTHUR JONES LOGIE

1907–1952

Dr. Arthur Jones Logie, of Miami, Florida, died suddenly on Friday, June 13, 1952.

He was born in New York City in 1907 and received his formal medical education at the Royal Academy of Edinburgh, Scotland, from which he was graduated in 1933. His postgraduate training was obtained in the Edinburgh Royal Infirmary; United Hospital, Port Chester, New York; Sea View Hospital for Tuberculosis, New York City.

He served as Medical Director of the Tuberculosis Division of the Florida State Board of Health from 1936 to 1941. He instigated the first mass X-ray screening of the people of this state for the detection of pulmonary tuberculosis. During the war he acted as Captain in the U. S. Public Health Service.

Dr. Logie was a Fellow of the Board of Internal Medicine and a member of various organizations relating to his professional activities.

PAUL RINGER

1881–1952

The cause of tuberculosis lost a valiant worker in the recent death of Dr. Paul Ringer who practiced medicine in Asheville, North Carolina, for forty years. He was a close student of tuberculosis and made numerous valuable contributions to the literature. In fact, his excellent paper on tuberculin in the Southern Medical Journal in 1918 was the "knockout blow" to the incautious and extensive use of this product in the treatment of the disease.

That Dr. Ringer's fellow-workers recognized his outstanding capacities is attested by his election to the presidency of his State Medical Society, Southern Medical Society, Southern Tuberculosis Conference, and to the office of vice president of the National Tuberculosis Association and his appointment to the Board of Directors of the North Carolina Sanatoria. He served in World War I.

Dr. Ringer was born in New York City in 1881; his father was a university professor and his mother, a musician. His early cultural interests he retained throughout his long life. He busied himself with civic affairs and led in Asheville Civic Music activities. He

was a man of many talents, rich in personality, and influential in the medical, religious, and cultural life of his area. His death will be mourned by many a patient, grateful for his considerate and effective service, and by many a citizen who appreciated the quality and work of the man.

ARTHUR KINGSBURY STONE

1861–1952

Dr. Arthur Kingsbury Stone, of Framingham, Massachusetts, died on July 31, 1952, in his ninety-first year. He was a native of Boston. He was educated at Framingham High School and Academy, and at Harvard College. In 1888, he received his medical degree from Harvard Medical School.

Dr. Stone was a pioneer in the fight against tuberculosis on both the local and the national level. He was chairman of a board of trustees appointed by the Governor to supervise the care of the tuberculous in Massachusetts. He was also a director of the National Tuberculosis Association.

He was interested in work at the House of the Good Samaritan, where he was at one time chief of staff, and in the activities of the Massachusetts Medical Society, of which he was treasurer from 1917 to 1931. Dr. Stone was also interested in other medical organizations of the State.

MANMOHANDAS B. SOPARKAR

1884–1952

Dr. Manmohandas B. Soparkar, former president of the Medical Section of the Indian Science Congress Association and Fellow of the National Institute of Sciences of India, died May 31, 1952, at the age of sixty-eight. He was graduated from Bombay University, worked as Chief Medical Officer for an Indian State.

Dr. Soparkar was interested in tuberculosis and did important bacteriologic work in this field at the Haffkine Institute. He later became assistant director and director of the Haffkine Institute where he continued his investigations on tuberculosis both in man and in animals.

He carried on bacteriologic work in numerous other diseases which were important in his country, and was the recipient of numerous medals and awards for his work.

THE AMERICAN REVIEW OF TUBERCULOSIS

ABSTRACTS

Edited by LAWRENCE B. HOBSON

VOLUME 67 MARCH, 1953 ABST. NO. 3

CLINICAL STUDIES, TUBERCULOSIS

Surgery for Tuberculosis.—Among the various surgical procedures available for the treatment of pulmonary tuberculosis, thoracoplasty and resection are the most effective and dependable. The latter is now developed to the point where diseased segments or lesser sections of the lung may successfully be removed. The benefits of these procedures depend on a wise selection of cases, adequate preparatory treatment with rest and antimicrobial therapy, highly skilled surgical technique, attentive postoperative care, and further periods of rest after recovery from the operation. In the approach to treatment, the first goal is the attainment of recovery which will be permanent. The immediate saving of time is of secondary importance to the avoidance of relapse. The progress of surgery and the availability of a number of specific drugs necessitate a reorientation of indications for various therapeutic procedures such as artificial pneumothorax and artificial pneumoperitoneum (Author's summary).—*Evaluation of developments in the surgical treatment of pulmonary tuberculosis, J. B. Amberson, Ann. Int. Med., September, 1952, 37: 482.*—(*T. H. Noehren*)

Surgery for Pulmonary Tuberculosis.—Between the years 1920 and 1948, total thoracoplasties were performed on 192 patients. Of these, 20 (10.5 per cent) died postoperatively, 64 (34 per cent) died later, 69 (35.9 per cent) are cured, 10 (5.4 per cent) are improved, 5 (2.7 per cent) are unimproved, and in 24 (12.6 per cent) the result is unknown. Only 37 (19.3 per cent) are alive eleven to thirty years after the operation. During the same period, partial thoracoplasties were performed on 154 patients. Of these, 6 (3.8 per cent) died postoperatively, 46 died later, 60 (38.9 per cent) are cured, 13 are improved, 5 are unimproved, and in 24 the result is unknown. Between the years 1949 and 1951, when streptomycin and *p*-aminosalicylic acid were available, partial thoracoplasties were performed on 49 patients. Of these, none died postoperatively, 2 died later, 37 are cured at present, 3 are improved, and in 7 the result is unknown. Between the years 1920 and 1928, 46 patients with mixed infection empyema underwent Bülau-drainage and partial thoracoplasty. Of these, 9 died postoperatively, 16 died later, 16 are cured, one is improved, and in 4 the result is not known. During the same period, 44 patients had paraffin plombage. Of these, 6 died postoperatively, 15 died later, 13 are cured, 2 are improved, one is unimproved, and in 7 the result is unknown. Of 139 patients who had extrapleural pneumonolysis before streptomycin was available, 5 died postoperatively, 20 died later, and postoperative complications occurred in 25. Of 61 patients with extrapleural pneumonolysis while receiving streptomycin, none died postoperatively, 2 died later, and postoperative complications occurred in 5. Cavernostomies were made for 34 patients. Of these, 4 died postoperatively, 4 died later, 18 are cured at present (4 of these had an additional thoracoplasty), 5 are improved, and in 3 the result is unknown. Between the years 1948 and 1951, 20 patients had lobectomies for tuberculosis. Of these, 2 died postoperatively, 2 died later, 12 are cured at present, 3 have tubercle bacilli in their sputum, and in one the result is unknown. During the same period, pneumonectomies for tuberculosis were performed on 20 patients. Of these, 3 died postoperatively, 4 died later, 12 are cured at present, and in one the result is unknown.—*30*

Jahre chirurgische Behandlung der Lungentuberkulose, W. Denk & R. Kühlmayer, Wien. klin. Wchnschr., August 29, 1952, 64: 655.—(G. C. Leiner)

Pulmonary Resection.—Cavities under an intrapleural or extrapleural pneumothorax should be treated with emergency pulmonary resection when the danger of perforation seems imminent. The signs that the cavity might break through into the pleural space were: a cavity remaining patent under a pneumothorax and situated within 4 mm. of the lung surface; progressive enlargement of such a cavity; repeated formation of fluid which became turbid; incomplete pneumonolysis for a peripheral cavity, followed postoperatively by fever and pleural exudate. If perforation of the cavity has already taken place, emergency resection should be performed whenever there is a reasonable chance of success. The following factors were prognostically unfavorable and constituted relative contraindications to surgery: (*1*) perforation several days old, with deterioration of the patient's condition and a fresh spread to the contralateral lung; (*2*) deterioration prior to the break-through of the cavity; and (*3*) bacterial resistance to streptomycin. A fresh spread to the homolateral side was no contraindication to pneumonectomy. Fever up to 104°F. responded spectacularly to resection. During 1950 and 1951, emergency resection was performed in 13 such cases; in 10 of these, perforation of the cavity seemed imminent, in 3 others it had already taken place. Four patients had pneumonectomy, 6 lobectomy, and 3 had segmental resection. There was one death following lobectomy when the bronchial sutures ruptured and a bronchopleural fistula developed; in a second case of postoperative bronchopleural fistula, surgical repair of the fistula and extensive thoracoplasty eventually gave a good result. The postoperative course was uneventful in 5 cases; 5 patients had a homolateral spread and subsequent thoracoplasty was necessary in 2 of them. The high incidence of these spreads is explained by the fact that resection was done in relatively fresh cases, recently subjected to the shock of pneumothorax, pneumonolysis, or perforation of cavity. In 4 of the 5 cases, the postoperative spreads have completely regressed; one has progressively improved. At the time of writing, 10 of the 13 patients could be considered well controlled roentgenographically, bacteriologically, and clinically.—*Treize cas d'exèreses d'urgence pour cavernes tuberculeuses perforées ou sur le point de se perforer dans la plèvre, Chalnot & Lochard, Semaine d. hôp. Paris (La Semaine médicale), September 18, 1952, p. 571.—(V. Leites)*

Excision in Primary Tuberculosis.—Four cases of irreversible lesions were caused by primary tuberculosis with rupture of lymph nodes, necessitating pulmonary resection including one pneumonectomy and 3 lobectomies. It is suggested that, after careful bronchography, resection of lymph nodes should be performed in certain cases of complicated primary infection to avoid definitive disturbances of ventilation with bronchial stenosis and bronchiectasis (Author's summary).—*Traitement chirurgical de certaines formes compliquées de la primo-infection de l'enfant, H. Métras, Rev. de la tuberc., 1952, 16: 569.—(V. Leites)*

Liver and Gallbladder Tuberculosis.—In a case of tuberculosis of the liver and gallbladder, there was radiographic evidence of a fistula between the gallbladder and the duodenum and a filling defect in the stomach which suggested a malignant tumor. Local tuberculosis of the liver is usually diagnosed at autopsy, but occasionally at laparotomy. The condition is said to occur most frequently in children and in various racial groups with little natural immunity to tuberculosis, such as Negroes and Igorots; but it is by no means restricted to these groups. In the vast majority of cases, tuberculosis is present elsewhere in the body, very few instances having been recorded in which the lesion of the liver was either the only one present or the oldest tuberculous lesion. The most common findings in tuberculous abscess of the liver, in order of their frequency, are fever, liver enlargement,

and chills. Jaundice is seldom encountered in this condition. The two main diagnoses to be differentiated from local tuberculosis of the liver are liver abscesses of other etiology and malignancy.—*Tuberculosis of the liver and gallbladder with abscess formation: A review and case report, S. A. Leader, Ann. Int. Med., September, 1952, 37: 594.*—(*T. H. Noehren*)

Caseous Pulmonary Foci.—In 15 resected lobes of lung segments containing single circumscribed caseous foci, the bronchus of the segment containing the focus or foci showed the following changes: (*1*) It was patent in 2 cases, opening into the caseous focus; the bronchial structure was destroyed by caseation. The caseous foci were "tuberculomas" in the process of liquefaction and the surrounding segment was nonaerated. Both patients had sputum positive for tubercle bacilli. (*2*) In 8 cases, the segmental bronchus ended blindly at the junction of the caseous mass; the surrounding parenchyma was airless. (*3*) In 5 cases, the pathologic changes involved the divisional bronchi, while bronchi leading to the caseous focus were permeable but distorted. The pulmonary segment was partly aerated. In 7 of 15 cases, considerable peribronchial adenopathy was present; in 3 cases, the adenopathy was in intimate contact with the segmental bronchus. In 9 of 14 cases, the junction of the segmental and lobar bronchus was the site of important changes in the submucosa: large areas of lymphoid infiltration containing giant cells were present. It is believed that the fibrous outer membrane and the bronchial stenosis cause the "exclusion" of the caseous focus. The fragility or the resistance of this double barrier promotes or inhibits liquefaction of the caseous material and emission of tubercle bacilli.—*État de la bronche contiguë aux foyers caséeux limités du poumon, F. Magnin, H. Perriol, & Feroldi, Rev. de la tuberc., 1952, 16: 559.*—(*V. Leites*)

Laboratory Diagnosis of Tuberculous Meningitis.—In 150 patients with tuberculous meningitis who were admitted to the hospital for treatment with streptomycin, the examination of cerebrospinal fluid included: (*1*) a test for glucose, using Benedict's solution; (*2*) Pandy's test of globulin; (*3*) a cell count; (*4*) microscopic examination for *Mycobacterium tuberculosis*; (*5*) culture; and (*6*) animal inoculation. Of the 150 cases, 131 (87 per cent) were definitely diagnosed by demonstrating tubercle bacilli in the culture or in the inoculated guinea pig. Of the remaining 19, acid-fast bacilli were seen in the smear in 7; histologic or bacteriologic evidence was obtained post mortem in 3; in 9 patients, all still alive, tubercle bacilli were not found in the smear, culture, or guinea pig although the diagnosis was supported by biochemical and cytologic examinations. There was no doubt of the value of a qualitative glucose determination as an immediate test. In only 6 of the 150 cases, a high cerebrospinal sugar failed to point out the diagnosis. Tubercle bacilli were seen on direct smears from 111 patients. The cell count and Pandy's test, although necessary and valuable, did not possess the same specificity as the test for glucose. The results of microscopy, culture, and guinea pig inoculation were all positive to the same degree, 69, 69, and 67 per cent, respectively. In 70 cases, both cultures and guinea pig inoculations were positive; in 19 the culture was positive and the guinea pig test was negative. In 21 the culture was negative and the guinea pig test was positive. In 25 both tests were negative. In 12 cases, tubercle bacilli could not be demonstrated at any time.—*Observations on the laboratory diagnosis of tuberculous meningitis, R. W. S. Harvey, Brit. M. J., August 16, 1952, No. 4780: 360.*—(*E. A. Riley*)

CLINICAL STUDIES. NON-TUBERCULOUS DISEASES

Sarcoid-like Histoplasmosis.—Sarcoid-like lesions were present in lymph nodes, lung, spleen, liver, and other organs in 3 cases of fatal histoplasmosis. The cause of death in every case was bilateral mycotic caseation of the adrenal glands. In the caseous material, periodic acid stains demonstrated huge numbers of *Histoplasma* organisms which could

not be identified in sections stained in the routine way. Although the possibility cannot be entirely excluded that these cases may represent the concurrence of two diseases, sarcoidosis and histoplasmosis, the evidence strongly suggests that they are instances of uncomplicated histoplasmosis. It is concluded that *Histoplasma capsulatum* may be an occasional cause of the clinicopathologic picture to which the name sarcoidosis has been given (Authors' summary)—*Histoplasmosis: Three fatal cases with disseminated sarcoid-like lesions, H. Pinkerton & L. Iverson, A. M. A. Arch. Int. Med., October, 1952, 90: 456.*—(*G. C. Leiner*)

Acute Interstitial Fibrosis of Lungs.—Acute diffuse interstitial fibrosis of the lungs is a disease of unknown causation characterized by sudden onset of cough, hemoptysis, dyspnea, cyanosis, and fever. Death is caused by cardiac failure or pulmonary asphyxia. Since the first description of this disease by Hamman and Rich, 10 additional cases have been reported. A 55-year-old white man was admitted with the symptoms of dyspnea, cough, and hemoptysis. The roentgenologic diagnosis was chronic bronchial disease with a superimposed acute pneumonitis in the left lung. Dypsnea and cyanosis increased, and the patient died. The anatomic diagnoses were: acute interstitial fibrosis of the lungs; fibrin within the pulmonary alveoli; fatty metamorphosis of the liver; chronic passive congestion of the liver; and undifferentiated carcinoma of the right lower lobe bronchus. The microscopic examination revealed: diffuse proliferation of young fibroblasts within the alveolar septa and in the perivascular and peribronchial regions, and filling of alveolar spaces with a fibrinous material. The disease may be due to a viral infection or due to a chronic pulmonary lymphedema.—*Acute diffuse interstitial fibrosis of the lungs, W. P. Callahan Jr., J. C. Sutherland, J. K. Fulton, & J. R. Kline, A. M. A. Arch. Int. Med., October, 1952, 90: 468.*—(*G. C. Leiner*)

Asthma in Childhood.—A series of 209 asthmatic children attended an allergy clinic during the years 1947 through 1951; 142 were boys, 67 were girls. In 84 per cent, symptoms appeared during the first seven years of life. A close relative had suffered from an allergic condition in 49 per cent of the cases. The two commonest associated allergic manifestations were eczema and rhinitis; the latter being difficult to separate from infectious rhinitis. Of the 209 cases, 57 (27 per cent) suffered or had suffered from eczema. Of these, 35 were boys and 22 were girls; 33 per cent of the girls and 24 per cent of the boys had asthma and eczema coincidentally. In 13 of the 35 boys and in 19 of the 22 girls, eczema was present after the age of three years. Positive specific skin reactions were obtained in 106 children (51 per cent), chiefly to inhalant antigens. Although 16 children were sensitive to pollens, ragweed sensitivity was not observed. One child was sensitive to milk protein. Clinical or radiologic evidence of pulmonary disease other than emphysema was obtained in 21 patients (10.5 per cent); 7 had bronchiectasis, 4 had calcified tuberculous foci, one had active tuberculosis, 4 showed increased hilar markings, one had bilateral cystic disease of the lung, one had lobular atelectasis, 2 had hilar fibrosis, and one showed changes due to congestion. Although 65 boys and 31 girls had had whooping cough, only 19 of the boys and 5 of the girls developed asthma with or immediately after that disease. Phenobarbital, 15 to 30 mg., once or twice daily, was given most patients for long periods. Many also received antihistaminic drugs. Ephedrine, 15 to 30 mg., was given to relieve the spasms, and children who were constantly wheezy received ephedrine regularly with phenobarbital. Children with specific skin sensitivity were desensitized if they were more than five years old. In some cases, allergens were removed from the child's environment. In addition to the 60 children whose tonsils and adenoids had been removed earlier, 47 underwent this operation upon advice from the clinic; 40 children had antral irrigations, and 8 had other intranasal operations. All children suffering from rhinitis were given decongestive drops. A total of 119 children (57 per cent) needed physiotherapy to correct defective

costal expansion, dorsal kyphosis, round shoulders, and contracted pectoral muscles. Only 14 children received psychiatric treatment because environmental disadvantages seemed to contribute to the frequency of their asthmatic attacks. Of 179 children who were followed for at least six months, 90 had no attack during the six months prior to the collection of these data, and 77 had markedly fewer attacks. Two children showed no improvement, and 10 had stopped attending the clinic. A total of 44 children have been free from attacks for more than one year.—*Asthma in childhood, C. B. S. Fuller, Brit. M. J., September 20, 1952, No. 4785: 636.*—*(E. A. Riley)*

Spontaneous Pneumothorax.—Spontaneous pneumothorax may be due to the rupture of (*1*) emphysematous blebs near pulmonary scars, (*2*) blebs in generalized pulmonary emphysema, or (*3*) congenital pulmonary cysts. Among other congenital anomalies which may cause a spontaneous pneumothorax may be a weakness of the elastic, subpleural and pleural tissue; small, invisible fistulas between alveoli and pleura may develop. The differential diagnosis between a spontaneous pneumothorax and a giant cyst may be extremely difficult. The chronic and the recurrent spontaneous pneumothorax may be treated by (*1*) production of pleurisy by injection of an irritant substance into the pleural cavity, (*2*) fluoroscopy and pneumonolysis or cauterization of the fistula, or (*3*) thoracotomy combined with pneumonolysis or artificial pleurisy, with suture of the lung fistula, with resection of the ruptured cyst, or with resection of the affected part of the lung. Among 7 cases of spontaneous pneumothorax, the lung re-expanded under conservative treatment in 3. One case of bilateral recurrent pneumothorax on basis of a cystic lung was not treated surgically because of the presence of an advanced malignant disease. Three patients with chronic and recurrent pneumothorax had thoracotomy and closure of the fistula, and remained well.—*Der Spontanpneumothorax, E. C. L. Jenny, Wien. klin. Wchnschr., September 26, 1952, 64: 765.*—*(G. C. Leiner)*

Pneumohemothorax.—An additional 3 cases of spontaneous pneumohemothorax increases the total reported to 56. The immediate aim of treatment is (*1*) to combat shock, hemorrhage, and anoxia by blood transfusions and administration of oxygen, (*2*) to prevent or counteract infection by antibiotic treatment intrapleurally as well as intramuscularly, and (*3*) to maintain the heart and mediastinum in their normal positions by repeated aspirations of blood and air. In the definitive treatment, a determined effort is made to promote complete re-expansion of the lung and thus prevent impairment of the pulmonary function. If repeated aspirations of the chest fail to release the lung, intrapleural instillations of streptokinase and streptodornase are employed to liquefy the blood coagulum and, if necessary, decortication is done.—*Spontaneous pneumohemothorax, E. L. Eidinger & E. H. Rubin, Canad. M. A. J., July, 1952, 67: 43.*—*(E. A. Riley)*

Hemothorax.—Twelve patients with traumatic hemothorax were treated early with intercostal closed catheter drainage and intrapleural injections of streptokinase and streptodornase. The enzymes, dissolved in 20 cc. of isotonic saline solution, were introduced through a catheter which was then occluded. After eight hours, suction at a vacuum of 6 to 8 cm. of water was applied by a Stedman pump to remove the liquefied material and to encourage re-expansion. The enzymes were most effective if treatment was begun immediately after active bleeding stopped. Treatment was given daily, the usual patient requiring more than one or two injections of the enzymes. The therapeutic results in all 12 patients were excellent.—*The treatment of hemothorax, with particular reference to the use of streptokinase and streptodornase, J. M. Miller & P. H. Long, U. S. Armed Forces M. J., July, 1952, 3: 1061.*—*(E. A. Riley)*

Streptodornase Toxicity.—The 28 patients studied received a total of 90 local instillations of streptokinase and streptodornase. Observations were made before treatment, immediately following, and then twice daily

until control levels were obtained. In 20 patients some type of reaction occurred and lasted for twelve to forty-eight hours after 44 of 67 treatments. There were 22 febrile reactions with temperature rises of 1.8°F. to 7.0°F. These were always associated with leukocytosis and the appearance of formed elements in the urine and occasionally with mild glucosuria and albuminuria. There were also 22 occasions when leukocytosis or increased numbers of urinary red and white cells were observed in the absence of fever. Red blood cell counts, hemoglobin concentrations, clotting and prothrombin times, cephaline flocculation tests, and erythrocyte sedimentation rates showed no significant change. No late toxicity was noted in 10 patients on whom additional observations were made from twenty-one to one hundred and twelve days after the last treatment.—*The systemic toxic responses of patients to treatment with streptokinase-streptodornase, W. N. Hubbard, Jr., J. Clin. Investigation, November, 1951, 30: 1171.*—(*S. Hadley*)

Chest Cage and Heart Failure.—Two patients had cardiac failure secondary to ineffective bellows action of the chest cage without significant pulmonary disease. In the first case, primary neuromuscular dysfunction existed; and in the second case, the patient had extensive calcified pleuritis which interfered with the mobility of the thoracic wall. In both patients the lesion reduced the vital capacity and maximum breathing capacity; ineffective alveolar ventilation was manifested by a high arterial partial pressure of carbon dioxide and arterial oxygen un-saturation. Both patients suffered from severe cardiac decompensation. These cases support other evidence that polycythemia and anoxia may lead to pulmonary hypertension which coupled with myocardial hypoxia may eventually result in cardiac failure.—*Cardiac failure secondary to ineffective bellows action of the chest cage, J. Feltman, W. Newman, A. Schwartz, D. Stone & F. Lovelock, J. Clin. Investigation, August, 1952, 31: 762.*—(*S. Hadley*)

Disturbed Diaphragmatic Movement.—Some patients complained of dyspnea or "inability to breathe" with pain or tightness over the anterior chest or abdomen but had no evidence of pulmonary or cardiac disease. Fluoroscopically, there were adequate diaphragmatic movements during quiet respiration; there was little or no diaphragmatic motion during forced respiration and, in some instances, there was slight paradoxical diaphragmatic movement. The absence of neurologic, thoracic, or abdominal lesions and the greater incidence of distress during periods of tension indicated that faulty breathing habits were responsible. Instruction in diaphragmatic breathing afforded a cure in each case. The syndrome, which may be called "disturbed diaphragmatic movement," probably is common, usually being mistaken for DaCosta's syndrome, cardiac neurosis, or anxiety state.—*Disturbed diaphragmatic movement, D. A. Ballantyne, New Zealand M. J., April, 1952, 51: 95.*—(*L. B. Hobson*)

Anoxia in Chronic Lung Disease.—Various investigators report different effects of acutely induced anoxia upon respiration and circulation. The authors studied the circulatory responses of 35 patients with pulmonary and cardiopulmonary disease to selected levels of oxygenation, higher and lower than room air. Particular attention was paid to achieving a "steady state" before measuring the cardiac output by the direct Fick method. Patients unable to reach a "steady state" were excluded. When patients with pulmonary disease were exposed to moderate anoxia, in most cases 16 volumes per cent, their cardiac output remained unchanged or increased. The pulmonary artery pressure increased by more than 5 mm. of mercury in a third of the patients and remained unchanged in the others. These results differ from those in normal persons. At least 3 factors may be important: (*1*) the observed tendency of the elevated pulmonary arterial pressures to fall toward initial levels as breathing of low oxygen continues; (*2*) the difference which may exist

between the response of the chronically anoxic and normal subject to acute anoxia; and (3) the significantly higher alveolar partial pressure of oxygen attained in these patients when compared to previous studies in normal animals and men.—*Effects of acute anoxia on the circulation and respiration in patients with chronic pulmonary disease: Studies during the "steady state", A. Fishman, J. McClement, A. Himmelstein, & A. Cournand, J. Clin. Investigation, August, 1952, 31: 770.*—(*S. Hadley*)

Mechanics in Emphysema.—Expiratory obstruction of a check-valve type in emphysema is suggested by the retarded expiratory air flow, even with strenuous muscular effort, despite unimpeded inspiration. Using the method of Neergaard and Wirz, determinations of air flow were made in normal and emphysematous patients, all of whom had either therapeutic or spontaneous pneumothoraces. Simultaneous measurements of pleural pressure made it possible to calculate the lung tension, the pulmonary pressure, i.e., the difference between lung tension and pleural pressure, and the resistance in the respiratory tract. In normal persons there was a direct proportion between lung size and lung tension. In the emphysematous patient, lung tension fell off rapidly despite persisting high pulmonary pressures. This resulted in the development of very high resistance. The airways collapsed, setting up a check-valve mechanism similar to that seen normally in the momentary collapse of the trachea as the glottis opens with cough. There is a tendency to develop such a check valve during health, but a variety of mechanisms exist to protect against it. In emphysema, however, lung tension is reduced and does not bear a direct relation to lung volume. This is probably the result of breakdown of the parenchyma which leaves the bronchioles unsupported and vulnerable to expiratory check-valve closure. The authors do not believe that there is valid evidence that the remaining parenchyma of the emphysematous lung is inelastic.—*Mechanics of air flow in health and in emphysema, H. Dayman, J. Clin. Investigation, November, 1951, 30: 1175.*—(*S. Hadley*)

Alveolar-Arterial Oxygen in Anemia.—Observations were made and compared in 11 normal persons who had 10.5 to 15.5 gm. of hemoglobin per 100 cc. and in 10 anemic patients with 2.0 to 8.9 gm. of hemoglobin. Alveolar and arterial oxygen tensions were measured and the alveolar-arterial oxygen tension gradients were calculated. The average gradient was 4.2 plus-minus 2.0 mm. of mercury in the normal subjects, as compared with an average gradient of 20.5 plus-minus 4.6 mm. of mercury in the anemic subjects. This difference is significant and can be explained on the basis of a normal admixture of venous blood, in which the per cent of oxygen saturation is lower than normal. Riley and Cournand found a value of 4.2 per cent for mean venous admixture with arterial blood. The results of these calculations are of the same magnitude with a value of 6.6 per cent.—*The alveolar-arterial oxygen pressure gradient in anemia, J. M. Ryan & J. B. Hickam, J. Clin. Investigation, February, 1952, 31: 188.*—(*S. Hadley*)

Lung Volume in Boys.—Data on the lung volume and its subdivisions (inspiratory capacity, expiratory reserve, and residual volume) were collected using 124 normal boys aged ten to seventeen years. They supply a basis for evaluating pulmonary function in diseases as well as establishing a range of variation for the normal child. By analyzing the results statistically using various physical measurements, it was found that height (H) or H^3 is the best single standard of reference. In the resting recumbent position the relative proportions of the total lung capacity occupied by the inspiratory capacity, expiratory reserve, and residual volumes were found to be 63.9, 17.0, and 19.1 per cent, respectively, in these boys. An effect of age on these values was suggested by relatively lower values for the expiratory reserve and residual volumes at thirteen and fifteen years of age.—*The*

lung volume and its subdivisions in normal boys 10–17 years of age, M. Morse, F. Schlutz, & D. Cassels, J. Clin. Investigation, April, 1952, 31: 382.—(*S. Hadley*)

Cardiac Rotation in Sarcoidosis.—A patient with Boeck's sarcoid of the lungs had unusual cardiac configuration and electrocardiographic changes indicating rotation of the heart. Primary cardiac involvement by sarcoid may or may not produce symptoms. Occasionally, the earliest symptoms are cardiac. The most frequent secondary cardiac manifestation of sarcoidosis is cor pulmonale caused by pulmonary fibrosis and hypertension. The cardiac rotation in this instance may be a rare secondary manifestation caused by pulmonary fibrosis and emphysema in the presence of mediastinopleuro-pericardial adhesions.—*Boeck's sarcoid with unusual cardiac configuration and electrocardiographic changes, J. Rubinstein & I. G. Kroop, Am. Heart J., August, 1952, 44: 313.*—(*G. C. Leiner*)

Bilharzic Cor Pulmonale.—A case of schistosomal heart disease, bilharzic cor pulmonale, was tentatively diagnosed because of the history. There had been adequate exposure to the cercariae; hematuria appeared within one year thereafter and persisted for eight years without any other known cause. The characteristic, though rare, cor pulmonale with aneurysmal dilatation of the major pulmonary arteries was seen in the angiocardiogram; other causes, such as hemolytic disease and atrial septal defect, were carefully excluded. A definite diagnosis was established by finding *S. haematobium* in the urine. An angiocardiogram, electrokymogram, ballistocardiogram, and twelve-lead electrocardiograms were recorded for the first time in this condition. This is the first reported case in which an antemortem diagnosis of bilharzial heart disease was made in this country.—*Schistosomal heart disease: bilharzic cor pulmonale, H. Vessell & J. A. Schak, Am. Heart J., August, 1952, 46: 296.*—(*G. C. Leiner*)

Pericardial Cysts.—Six patients with coelomic cyst of the pericardium were operated upon without complications. The patients ranged in age from fourteen to sixty-two years and most were asymptomatic. One patient complained of slight pericardial pain and another of shortness of breath and backache. The cysts ranged in size from that of a walnut to that of an orange. The roentgenographic appearance was that of a rounded shadow of uniform density in the anterior mediastinum; more frequently in the right cardiophrenic angle. Diagnostic pneumothorax revealed the lack of connection of the shadow to the lung. A positive preoperative diagnosis in this type of case is seldom possible. The differential diagnosis should include: neurofibroma, hydatid cyst of the lung, cystic lymphangioma, bronchogenic cyst, solid or cystic teratoma, hamartoma, thymoma, diaphragmatic hernia, and hydatid cyst of the liver. Pneumoperitoneum is of distinct help in differentiating the cyst from the latter 2 possibilities. Surgical excision is recommended as the treatment of choice for coelomic pericardial cysts. The strongest argument for surgery is the impossibility of distinguishing preoperatively these benign cysts from other lesions which are malignant or potentially malignant.—*Quistes celómicos y divertículos del pericardio, Victor Armando Ugon, Hoja tisiol., December, 1951, 11: 303.*—(*F. Pérez-Pina*)

Isolated Intrathoracic Nodules.—Mass chest roentgenographic surveys, originally intended to detect early pulmonary tuberculosis, have also revealed other intrathoracic lesions in apparently healthy people. Among a varied assortment of symptomless intrathoracic conditions are isolated, well-defined nodules, often small, which may offer difficulty in diagnosis and therapy. If such a nodule is inflammatory or a benign tumor, there is usually no urgency in recognizing and treating it; if it is an early carcinoma, incorrect diagnosis and improper management may prove disastrous. Of 25 silent circumscribed nodules in apparently normal people, 6 (25 per cent) were bronchogenic carcinomas; 7, tuberculomas; 2, bronchial adenomas; 2, hamartomas; 2, metastatic carcinomas; 1, fibroma; 1 pericardial cyst; 1 arteriovenous aneurysm; and 3 granulomas

of undetermined etiology. There are no satisfactory criteria to differentiate the 25 per cent which were primary bronchogenic carcinomas. With few exceptions, asymptomatic isolated intrathoracic lesions are correctly treated only with prompt surgical exploration if an exact preoperative diagnosis is impossible or if the presence of a malignant tumor cannot be excluded with certainty.—*The diagnosis and management of asymptomatic isolated intrathoracic nodules, S. E. Wolpaw, Ann. Int. Med., September, 1952, 37: 489.*—(*T. H. Noehren*)

Lymphangitic Carcinoma.—Two patients with primary gastric carcinoma and generalized carcinomatous permeation of the pulmonary lymphatics initially had severe, progressive dyspnea which overshadowed other evidence of malignant disease. Generalized cancerous permeation of the pulmonary lymphatics is much less common than nodular, solid, or linear metastases, but it causes a more striking clinical picture of extreme, unrelenting dyspnea. The usual primary sites are the stomach, bronchus, breast, prostate, and pancreas. Roentgenographically there are: (*1*) linear striations converging radially toward the hilum, (*2*) granular or nodular mottling, (*3*) reticulation, often very well shown at the periphery, and (*4*) hilar glandular enlargement. There may be effusion, atelectasis, or congestive heart failure. Symptoms such as weight loss, weakness, and anorexia, which might in other circumstances be attributed to malignancy, can be regarded as secondary to the distress induced by severe dyspnea. Although the onset of symptoms is often insidious, it may be rapid and dramatic. It may be precipitated by, or occur simultaneously with, an upper respiratory infection. The physical signs usually are few in proportion to the degree of dyspnea, with only scattered rales or rhonchi, poor respiratory excursion, and sometimes pleural effusion. Asthmatic wheezing is common. Right heart failure may be evident if pulmonary endarteritis has developed with resulting pulmonary hypertension. The diagnosis is suggested by the combination of severe dyspnea, few signs of congestive heart failure, and the characteristic radiographic picture. Marked weight loss is suggestive, and the complete failure of all treatment affords valuable confirmation. Probably dissemination is retrograde along the pulmonary lymphatics after involvement of the hilar nodes. Gastric neoplasm is so often the primary tumor, perhaps because the lymphatic connections between the regional lymph nodes of the stomach and of the lung are comparatively short. The obliterative changes in the pulmonary artery are due to a combination of endarteritis and thrombosis with subsequent organization.—*Lymphangitis carcinomatosa, G. N. Chandler & M. Telling, Brit. M. J., September 20, 1952, No. 4785: 639.*—(*E. A. Riley*)

Arteriovenous Fistula of Lung.—The diagnostic triad of arteriovenous fistula of the lung is cyanosis, clubbing of the fingers and toes, and polycythemia; however, it may occur without any symptoms. A survey chest roentgenogram of an asymptomatic 48-year-old white man showed a round shadow in the left lung field adjacent to the cardiac apex. Lateral tomography revealed an area of opacity in the lingula and two band-like densities extending from the opacity toward the hilum. The diagnosis of an arteriovenous fistula was considered. A segmental resection of the lingula, which contained the fistula, was done. The patient made an uneventful recovery.—*Asymptomatic arteriovenous fistula of the lung: Report of a case with surgical cure, T. J. Talbot & J. J. Silverman, A. M. A. Arch. Int. Med., October, 1952, 90: 569.*—(*G. C. Leiner*)

Radioactive Gold for Effusions.—Radioactive colloidal gold was placed in the appropriate body cavity of 16 patients with malignancies involving the chest and abdominal cavities. The primary objective was to reduce fluid accumulation; a secondary objective was to relieve pleuritic or abdominal pain. Radioactive gold was considered appropriate because (*1*) the half-life of Au. 198 is 2.7 days, (*2*) it emits a moderately energetic beta particle and 2 soft gamma rays, about

90 per cent of the ionization occurring in the first millimeter of exposed serosal surface; (*3*) its particulate size is such that almost none enters the vascular and lymphatic systems. The cases treated included 6 metastatic breast carcinomas, 2 cystadenocarcinomas with ascetis, one each of lymphoblastoma of the mediastinum, generalized Hodgkin's disease, adenocarcinoma of the rectum, and rhabdomyosarcoma of the chest wall. The therapeutic response was, in general, encouraging, especially in those patients treated before they were moribund. Four patients obtained marked relief, with relief of pain and little or no fluid accumulation. One of these patients, however, was relieved for only two and one-half weeks, when he died. Four patients experienced moderate relief, the intervals between taps being considerably lengthened; 2 of these patients are alive and no taps have been necessary for two and one-half and three and one-half months, respectively; another still has considerable chest pain. Four patients responded only slightly, with some reduction in the rate of fluid accumulation and relief of pain; 3 of them were moribund when treated. Of the remaining 4 patients, one obtained relief, another was treated following pneumonectomy as a prophylactic measure and is difficult to evaluate, another had pulmonary tuberculosis as well, and the last died only five days after therapy. It seems that the intracavitary use of radioactive colloidal gold is a valid radiotherapeutic procedure, but it has not yet been shown to prolong life or destroy malignant tissues.—*The use of radioactive gold, E. R. King, D. W. Spicer, W. Dowda, M. A. Bender, & W. E. Noel, Am. J. Roentgenol., September, 1952, 68: 413.*—(*T. H. Noehren*)

Chest Radioautography.—"Chest radioautography" is a new term which means the recording of radioactivity present in the lungs and pulmonary structures. After administration of a radioactive isotope, the patient's chest is placed in contact with an ordinary holder containing conventional roentgenographic film. The method is practical and useful, as in the case presented, in making the differential diagnosis of pulmonary lesions as well as in locating metastatic lesions in the chest. A 37-year-old white woman was referred for roentgenographic therapy with a diagnosis of papillary adenocarcinoma of the thyroid. She had previously been in a tuberculosis sanatorium for pulmonary lesions, apparently fibrocalcareous and distributed throughout both lungs. Her present difficulty began with right vocal cord paralysis; a mass incompletely removed from her neck was a carcinoma. Extensive lesions throughout both lungs showed no real change as compared with those seen in films taken at the sanatorium twelve years earlier. Roentgenograms did not reveal whether any of the densities were metastatic lesions from the thyroid. Six days after the administration of I^{131}, the patient was placed supine on a 14- by 17-inch cassette containing roentgenographic film. After one hour, the radioautograph was processed and the photographic effect of the radioactivity present in the pulmonary metastases was clearly shown. —*Chest radioautography, W. M. Loehr, Am. J. Roentgenol., September, 1952, 68: 355.*—(*T. H. Noehren*)

Rapid Chest Photofluorography.—A chest photofluorogram can be finished in one minute by a process which employs the Land Polaroid photography principle. Small objects of high density, such as wire mesh, show details in these photofluorograms approaching those obtained with conventional 70 mm. film. The short latitude of the Polaroid photographic material in the dark gray and black shades makes it more difficult to record small, faint shadows like those in minimal tuberculosis. Infectious tuberculosis, cardiac and aortic lesions, diaphragmatic abnormalities, and mediastinal tumors are clearly depicted. Rapid photofluorography has many advantages for the screening of hospital patients. If a photofluorographic unit of this type were near the main roentgenology department, the photofluorograms could be interpreted promptly; patients with suspicious lesions could have conventional roentgenographic procedures per-

formed immediately before admission of the patient to the ward. Besides reducing return visits to the roentgenology department, this method would reveal valuable information which frequently is not now available until after operation, if patients are admitted to surgery. Improvements in the optical system and recording media may adapt this process for some other uses.—*A method of rapid chest photofluorography, H. S. Weens & A. A. Rayle, Am. J. Roentgenol., September, 1952, 68: 457.*—(*T. H. Noehren*)

ANTIMICROBIAL THERAPY

Early Results with Isoniazid.—A series of 66 patients with pulmonary tuberculosis were treated with isoniazid, 4 mg. per kg. daily for three or four months. All of the patients had cavitary disease, bilateral in 42 and unilateral in 24; 59 had been or still were being treated with collapse therapy; 57 had received streptomycin and *p*-aminosalicylic acid (PAS). Most patients experienced very early a sense of well-being and increased vitality. Their appetites improved, and weight gains were often considerable. Most patients had no fever and little cough before the start of therapy so that isoniazid's effect on these symptoms could not be evaluated. The erythrocyte sedimentation rate dropped considerably in two-thirds of the cases. The quantity of sputum decreased in 46 cases and expectoration ceased in 12. Of the 66 patients, 43 were positive by direct smear, 14 by concentrate, and 3 by gastric lavage. In 35 patients, sputum and gastric contents became negative by direct smear and concentrate. Roentgenographic improvement was seen in 20 patients (30 per cent); progression of disease was present in one case, and there was no change in 45. Roentgenographic improvement consisted in diminution of the extent and density of homogeneous shadows; disappearance of post-hemorrhagic spreads; diminution in the size of some cavities; and thinning of cavity walls, leaving sometimes only a very fine outline. The isoniazid was generally well tolerated. Transient dizziness was noted in some cases at the beginning of treatment; 5 cases of constipation and one case of disturbed urination responded well to prostigmine. In 5 cases of tuberculous meningitis, of which 3 were relapses and none of which responded to streptomycin and PAS, oral and intrathecal isoniazid began to show promising results.—*Premières observations cliniques de malades traités par le Rimifon, J. Morin, Rev. de la tuberc., 1952, 16: 564.*—(*V. Leites*)

Isoniazid for Pulmonary Tuberculosis.—Since March, 1952, approximately 100 patients have been treated with isoniazid in a Swiss sanatorium for more than three months. In the beginning the daily dose of isoniazid was 5 mg. per kg.; this was soon increased to 10 or 12 mg. per kg. The higher dosage was well tolerated and was necessary to obtain amelioration in some cases. Later the dosage was again reduced to 4 to 8 mg. per kg. but *p*-aminosalicylic acid (PAS) was also given orally or intravenously. In some cases, the combined use of isoniazid, PAS, and streptomycin produced such favorable results as to suggest synergism. The clinical effects of isoniazid alone were: (*1*) increased appetite at the start of treatment; (*2*) euphoria, mostly due to psychological factors; (*3*) gain in weight, most marked during the first month; and (*4*) reduced volume and frequency of expectoration, purulent sputum becoming white and mucoid; in 15 patients, expectoration ceased completely. Most of the patients were afebrile at the start of treatment; in patients with slight fever, normal temperatures were gradually reached but the results did not differ from those obtained with bed rest alone. Of 42 patients who had positive bacteriologic findings at the start of treatment, the sputum and gastric contents became negative in 28 per cent as evidenced by ten to twelve cultures. These results were sometimes, but not always, accompanied by roentgenographic improvement. Isoniazid resistance developed after four or five weeks and increased rather rapidly. The disappearance of bacilli from the sputum could not be correlated with the emergence of resistance; sputum conversion and roentgenographic improvement often occurred after

isoniazid resistance had been demonstrated. Roentgenograms showed that: (*1*) old cavities with rigid walls were not influenced; (*2*) in some fresh cavities which developed within an area of infiltration, good results were observed; the walls became thinner and a linear contour developed; (*3*) cavity walls were distorted and some fresh cavities disappeared completely, even on tomography; (*4*) there was little or no effect on nodular and infiltrative lesions, especially on those of longer standing; (*5*) hilar adenopathy in primary tuberculosis regressed in a relatively short time but an increased tendency to fistula formation was noted; and (*6*) some roentgenographic progression was observed during treatment. Isoniazid is no substitute for the other accepted methods in the treatment of tuberculosis but during the three-month period several otherwise hopeless patients became eligible for pneumothorax, thoracoplasty, and lobectomy. Of the patients, 25 per cent improved roentgenographically because of the isoniazid; 8 per cent became worse; and no change occurred in 67 per cent. The erythrocyte sedimentation rate fell in all cases, frequently to normal. Only one case of anemia was seen but moderate leukocytosis with toxic granulations and slight eosinophilia was a frequent occurrence. Coagulation time was increased in one-third of the cases, while bleeding and prothrombin times remained normal. At the start of treatment with isoniazid, several hemoptyses occurred but later became infrequent. There were some cases of intractable epistaxis, and considerable hematuria appeared in one case of relatively well-stabilized renal tuberculosis. The recent studies of Berblinger seem to confirm the hemorrhagic properties of isoniazid.—*Notes sur l'isonicotinylhydrazide (INH), T. Wacker & E. C. Bonard, Semaine d. hôp. Paris, (La Semaine médicale), September 18, 1952, p. 583.—(V. Leites)*

Isoniazid Effect on Nucleotidases.—Diphosphopyridine nucleotide (DPN) is cleaved at the nicotinamide-ribose linkage by nucleotidases from various sources. Nicotinamide antagonizes such cleavage by beef spleen nucleotidases by competing with water for the diphosphopyridine-enzyme complex and thus preventing the disruption of the nicotinamide-ribose linkage. *Neurospora* nucleotidase is antagonized only by high concentrations of nicotinamide which simply compete with DPN. Isoniazid (isonicotinic acid hydrazide), structurally related to nicotinamide, is approximately ten times as effective as the latter in inhibiting the beef spleen enzyme, a concentration of 1.3×10^{-4} M isoniazid causing 50 per cent inhibition. Nucleotidases from the brain or spleen of beef and lamb are about equally sensitive; nucleotidases from the corresponding tissues of rat, mouse, and rabbit are relatively insensitive to isoniazid but are equally sensitive to nicotinamide. Nucleotidases from *Neurospora* are resistant to inhibition by both isoniazid and nicotinamide. Isoniazid as an analogue of nicotinamide may replace the latter in the DPN formed under the influence of some nucleotidases and this may explain its antimicrobial action.—*The action of nicotinamide and isonicotinic acid hydrazide on diphosphopyridine nucleotidases, L. J. Zatman, S. P. Colowick, N. O. Kaplan, & M. M. Ciotti, Bull. Johns Hopkins Hosp., September, 1952, 91: 211.—(L. B. Hobson)*

Tuberculous Meningitis.—During the period of October, 1948, to May, 1951, 26 patients with tuberculous meningitis were treated. In 10 of the patients, miliary tuberculosis was also present. Of the 26 patients, 14 survived; 12 have completed the treatment and made a good recovery; the prognosis for the 2 who are still being treated is good. Because relapses are common after brief courses of treatment, it was planned to give intramuscular streptomycin daily for six months and supplement it with intrathecal injections on approximately 75 per cent of the days. The intramuscular dose for an adult was 1 gm; and for children, 0.5 to 1 gm., according to age. Among 12 patients who have completed the course, intramuscular streptomycin was given an average of ten days more than the six months. Relapse occurred in 5 of the 10 patients whose treatment was terminated before six

months. The survivors received an average of 144 intrathecal injections of 50 to 100 mg. Deafness was the most serious toxic manifestation, occurring more frequently among those treated with dihydrostreptomycin alone than among those treated with part dihydrostreptomycin and part streptomycin calcium chloride. The prolonged use of dihydrostreptomycin is no longer justifiable. The sugar content of the cerebrospinal fluid was the most valuable prognostic guide and treatment had to be continued until the sugar exceeded 50 mg. per 100 ml. A falling sugar concentration during treatment was a bad sign. The chloride content of the cerebrospinal fluid, although less reliable than the sugar concentration, reflected the changes in the clinical state of the patient in general and followed the same course as the sugar. In assessing progress during treatment, the protein content and leukocyte counts of the cerebrospinal fluid, although additional aids, were not accurate and constant. Neither became normal so long as intrathecal streptomycin was given, and they remained abnormal longer than the other constituents after treatment was stopped. Streptomycin injected into the normal thecal space caused an immediate pleocytosis and outpouring of protein into the cerebrospinal fluid. This congestion, with the tuberculous exudate already there, may have caused a spinal block. A few days rest often led to its resolution; when resumed, the intrathecal dose was reduced from 100 to 50 mg. for adults and from 50 to 25 mg. for children. When the spinal obstruction persisted, cisternal injection was used. This sometimes caused annoying and potentially dangerous reactions including burning pains in the head, face, and neck, as well as drowsiness and an acute vestibular disturbance. Repeated cisternal streptomycin injections caused hemorrhage into the subarachoid space. Of the 10 patients with tuberculous meningitis and miliary tuberculosis, 7 had choroidal tubercles; 5 of these patients survived. No tubercles were seen in the 16 cases of meningitis without miliary disease. During the treatment of the 7 patients, only slight changes occurred in the tubercle during a period of many months. These lesions cannot be said to be healed but a phase of arrest or healing has been reached.—*Tuberculous meningitis, some aspects of its treatment, A. R. Somner, Brit, M. J., August 16, 1952, No. 4780: 356.*—(*E. A. Riley*)

Streptomycin for Ambulant Tuberculous Patients.—A series of 24 patients were selected as having persistently positive sputum without other signs of clinical activity. Tomography was done, when necessary, to exclude gross cavitation. In many cases, bronchoscopy was also performed to exclude tuberculous disease of the larger bronchi as the cause of the positive sputum. The expectorated tubercle bacilli were sensitive to 1 unit of streptomycin per ml. before treatment. All of the patients were living comparatively normal lives and no change was made in their normal routine during treatment. At first, each patient received 1 gm. of streptomycin daily intramuscularly for ninety days; *p*-aminosalicylic acid (PAS), 14.1 gm. daily, was given concurrently. The streptomycin dose has now been reduced to 1 gm. twice weekly for 26 doses. Among 7 men, aged nineteen to forty-two, who had chronic bilateral fibrotic disease too extensive for surgery, 3 have remained sputum-negative. Of 6 patients with some collapse measure, 4 had slight disease in the opposite lung; 5 now have consistently negative sputum. One man expectorated tubercle bacilli intermittently for seven months but has produced none during the following six months. All of the 4 patients who had had thoracoplasties have remained negative since chemotherapy. Among 7 patients with localized unilateral or bilateral fibrotic disease was one who had definite cavitation but refused surgery; 3 of these have produced one positive sputum since chemotherapy, but subsequent tests have been negative; one has been consistently negative; and one, although negative earlier, produced a positive sputum after five months and has since dropped out of the investigation. Of the 4 patients in the entire series whose sputum was converted and subsequently became positive again, 3 received

intermittent streptomycin treatment to a total of only 26 gm. Thus, 14 of the 24 patients have produced no bacilli during and since treatment. These men and women were working and living normal active lives during treatment. No toxicity to streptomycin was noted; PAS has been well tolerated, producing fewer symptoms than in bed patients. Only one patient had diarrhea due to PAS, necessitating a short period away from work. In no instance have the tubercle bacilli developed resistance to 1 unit of streptomycin per ml., and there has been no clinical evidence of deterioration. There was little change in the weight, sense of well-being, erythrocyte sedimentation rate, temperature, or roentgenographic findings during the period of study. In selected cases, the social and medical disadvantage of an infectious sputum can be prevented without interfering with the usual routine of life.—*Streptomycin for the chronic ambulant tuberculous patients, O. Clark, Brit. M. J., September 20, 1952, No. 4785: 644.—(E. A. Riley)*

Cortisone and Tuberculous Meningitis.—Since the addition of cortisone to a streptomycin regimen for tuberculous meningitis might prevent the formation or effect the dissolution of tuberculous exudate, a case of tuberculous meningitis was treated with cortisone and intramuscular streptomycin. The partial resolution of a lumbar subarachnoid block obtained was attributed to cortisone therapy. Within forty-eight hours of cortisone administration, the patient, who had been moribund, showed marked clinical improvement, which has persisted with apparent remission of her disease for a period of seven months following its onset.—*The treatment of tuberculous meningitis with cortisone and streptomycin. S. J. Shane, R. A. Clowater, & C. Riley, Canad. M. A. J., July, 1952, 67: 13.—(E. A. Riley)*

Cortisone and Tuberculosis.—Cortisone was given to one patient with scleroderma and to another with disseminated lupus erythematosus. The first patient developed a tuberculous abscess of the axilla with involvement of the shoulder joint shortly after treatment. The second had an apparently arrested pulmonary tuberculosis which was reactivated with miliary dissemination and, subsequently, meningitis. There is a definite risk that corticotropin or cortisone will activate tuberculosis. It is inadvisable to give these hormones to patients with active or quiescent tuberculosis unless there are clear-cut nontuberculous indications, in which event streptomycin and *p*-aminosalicylic acid should be given and probably continued for several months after hormone therapy.—*ACTH, cortisone and tuberculosis, A. W. Capon, Canad. M. A. J., July, 1952, 67: 46.—(E. A. Riley)*

Acquired Multiple-Drug Hypersensitivities.—A tuberculous patient who had no history of definite allergy was given streptomycin and sodium *p*-aminosalicylic acid (PAS) daily. Three weeks after starting therapy, the patient became briefly febrile with a slight headache. One week later she again became febrile, had nausea, vomiting, dyspnea, and pain in the right side of her chest. The next day her leukocyte count was 44,000 with eosinophilia, a generalized maculopapular rash appeared, and her urinary output decreased. Facial edema with large, tender posterior cervical lymph nodes and oral ulcers developed although the antituberculous drugs were discontinued. The rash, which became vesicular and later hemorrhagic, was made worse by sunlight. After a period of mental confusion with evidence of liver and kidney damage, the patient recovered. There was no extension of the pulmonary lesions. Subsequently, the patient reacted strongly to patch tests with 5 per cent sodium PAS, 1 per cent *m*-aminophenol, streptomycin, and dihydrostreptomycin. Seven months after this episode the patient was given penicillin for an acute effusion after a thoracoplasty. The antibiotic had to be stopped when a widespread punctate rash appeared. Besides stopping PAS as soon as any unexpected fever occurs, routine patch testing should be carried out with other drugs, particularly those of the aminophenol group,

preferably using a 1 per cent solution of each.—*Acquired hypersensitivity to sodium PAS, streptomycin, and penicillin, B. Jeffery, P. Borrie, & N. Macdonald, Brit. M. J., September 20, 1952, No. 4785: 647.*—(*E. A. Riley*)

Streptomycin and Isoniazid.—In order to study the *in vitro* effect of streptomycin in association with isoniazid, tubes containing 4 cc. of Dubos medium and decreasing concentrations of the 2 drugs were inoculated with *M. tuberculosis* H37Rv. An inhibitory effect was seen in the tube which contained 0.05 γ of isoniazid and in the tube containing 0.5 γ of streptomycin. In a third series of test tubes, various concentrations of a mixture of streptomycin and isoniazid in a proportion of 10:1 were added to 4 cc. of Dubos medium. An inhibitory effect was obtained with 0.2γ of streptomycin and 0.02 γ of isoniazid. This is considered to be merely an additive effect and not a true syngerism like the combined action of isoniazid and *p*-aminosalicylic acid.—*Action combinée in vitro de l'I.N.H. et de la stremptomycine sur le baciile de Koch, M. Aitoff & A. Salas, Ann. Inst. Pasteur, August, 1952, 83: 273.*—(*V. Leites*)

Iodo-chloroxyquinolin and Streptomycin.—After having demonstrated a favorable effect of iodo-chloroxyquinolin on experimental tuberculosis of guinea pigs, the combined action of this drug with ineffective doses of streptomycin was studied. Forty animals were divided in groups of 10 each and infected with 10 mg. of virulent human bacilli; *Group I* served as untreated controls. Treatment was started eighteen days after infection in the 3 other groups: *Group II*, 4 mg. of streptomycin daily; *Group III*, 8 mg. of an emulsion of iodo-chloroxyquinolin daily; *Group IV*, 4 mg. of streptomycin and 8 mg. of iodo-chloroxyquinolin daily. When autopsied, animals which had received combined therapy with iodo-chloroxyquinolin and streptomycin showed such regression of tuberculosis as compared with the other groups that a synergistic effect is indicated. No lesions were found in lungs, liver, and spleen of these animals on or after the twenty-second day of treatment. It is probable that treatment of human tuberculosis will benefit from these findings.—*Effet remarquable sur la tuberculose experimentale du cobaye de l'association de l'iodochloroxyguinoleine avec une dose subactive de streptomycine, F. Tison, Ann. Inst. Pasteur, August, 1952, 83: 275.*—(*V. Leites*)

Streptomycin Dependence.—Strains of dihydrostreptomycin-dependent bacilli were isolated from 2 patients treated with this drug. Both strains showed more abundant and more rapid growth on a medium containing dihydrostreptomycin than on the control medium. This property persisted in the course of several successive transfers. Guinea pigs were infected with bacilli growing on Youmans' medium containing 50 to 100 γ per cc. as the second and third transfers. The animals were divided into two groups: one group received daily injections of the same brand of dihydrostreptomycin as was used for the patients and for the culture medium; the other group served as untreated controls. At autopsy, both groups of guinea pigs showed classical experimental tuberculosis with hepatic, splenic, and pulmonary involvement. No difference in pathology was observed between the treated animals and the controls.—*La tuberculose experimentale du cobaye a bacilles streptomycine-dependants, J. Brun, J. Viallier, & J. C. Kalb, Ann. Inst. Pasteur, August, 1952, 83: 270.*—(*V. Leites*)

Streptomycin Resistance.—Many patients are admitted to the French Students Sanatorium with early lesions discharging few tubercle bacilli. Thus, in 60 per cent of the cases, streptomycin sensitivity can be determined only after the number of bacilli available has been increased, using organs of guinea pigs inoculated with sputum cultures. In spite of the delay, as compared with direct cultures, this technique is preferred because abundant bacterial growth is essential for reliable results. In order to determine the sensitivity with the least possible delay, streptomycin media are made as favorable as possible for the growth of

tubercle bacilli. Liberal oxygenation of liquid media by placing test tubes in a very inclined position makes it possible to obtain valid results four days after inoculation or one month after starting the primary culture. The culture time must be individualized according to the behavior of each strain.—*Procédé d'accélération de la determination de la streptomycino-resistance pour les produits paucibacillaires, A. M. Schlesinger, Rev. de la tuberc., 1952, 16: 478.—(V. Leites)*

Chemotherapy of Pulmonary Infections.—Before starting chemotherapy of various pulmonary infections such as purulent bronchiectasis, lung abscess, and infected pulmonary cysts, the sensitivity of the causative organisms to different chemotherapeutic agents was determined by a simple agar diffusion method, using direct sputum inoculation. The sensitivity of bacteria from purulent particles of sputum was determined for penicillin, streptomycin, chloramphenicol, aureomycin, and terramycin; the chemotherapeutic agent was chosen in accordance with these assays. The clinical results in 50 cases of various pulmonary infections were definitely superior to those obtained before introduction of this method.—*L'interêt du choix d'antibiotiques adoptés dans le traitement des suppurations broncho-pulmonaires, A. Duroux, P. Jarniou, & R. Celerier, Presse méd., August 30, 1952, 60: 1159.—(V. Leites)*

LABORATORY STUDIES

Effect of Sodium Hydroxide on Tubercle Bacilli.—The digestant most commonly used to obtain tubercle bacilli in pure culture is sodium hydroxide; unfortunately, this kills the bacilli. Sufficient sodium hydroxide was added to dilute suspensions of human tubercle bacilli to give a final concentration of 2 per cent. These mixtures were kept as usual at 37°C. and samples were removed immediately and at thirty and sixty minutes after adding the alkali. After they had been neutralized, aliquots of each sample were planted on five plates containing oleic acid medium for colony counting. Undigested but incubated control suspensions to which hydrochloric acid was added were likewise plated. Colony counts after forty-two days of incubation showed constant results. Incubation at 37°C. for one hour had no appreciable effect on the viability of the bacilli. Exposure to sodium hydroxide for five minutes reduced the count to 56 per cent of the controls. After thirty minutes of digestion, the average survival rate was 28 per cent; and after sixty minutes it had dropped to 11 per cent. To determine whether sputum protects tubercle bacilli, sodium-hydroxided-digested, neutralized, negative sputum was used as the diluent without slowing the bacterial destruction. Sodium hydroxide affects tubercle bacilli like other injurious chemical and physical agents; it kills most bacilli during the first unit of time while the few survivors often are killed only after prolonged exposure. These survivors do not yield cultures of higher resistance than the original strain. When tubercle bacilli are too few to be seen in stained preparations, it is unlikely that many will survive the action of sodium hydroxide. Reports of "negative" cultures should be interpreted cautiously.—*Toxic effect of sodium hydroxide on tubercle bacilli, D. Yegian & V. Budd, Am J. Clin. Path., May, 1952, 22: 456.—(J. S. Woolley)*

Cold Staining of Tubercle Bacilli.—While investigating the effect of surface-active substances, such as Tween 20, Tween 80, Antarox A-400, on tubercle bacilli, it was noted that the addition of these detergents during the Ziehl staining procedure made it possible to stain the bacilli at room temperature. Fuchsin was used in somewhat higher concentration than is usual, namely, 4 gm. per 300 cc. The detergent was added in a proportion of 10 drops per 100 cc. When fuchsin was used without the detergent and no heat was employed, 80 per cent of acid-fast bacilli were stained blue; 15 to 20 per cent were stained pale red, the granulations being poorly visualized. When pure fuchsin was replaced with a mixture of fuchsin and surface-active substance, the previous proportion was reversed: 90 per cent of bacilli were stained bright red

and granulations were well visualized; 10 per cent of bacilli appeared blue. Nonacid-fast germs were stained blue with the fuchsin-detergent mixture.—*Nouvelle application de l'usage des corps tensio-actifs en physiologie bactérienne; Coloration á froid des bacilles tuberculeux, J. Desbordes, E. Fournier, & C. Guyotjeannin, Ann. Inst. Pasteur, August, 1952, 83: 268.—(V. Leites)*

Rapid Culture of Tubercle Bacilli.—Sputa from 149 cases of known or suspected pulmonary tuberculosis were examined by direct smear, by smear of concentrate, and by a rapid culture method, using Long's liquid medium. To this liquid medium, sulfathiazole was added to a final concentration of 1:5,000. Three tubes of this liquid were inoculated with each sample of sputum. After two, four, and six days of incubation, smears of the centrifuged sediment were made and examined for acid-fast bacilli. Of the 149 sputa, 60 (40.2 per cent) were positive on direct smear or concentrate, while 92 (63.7 per cent) were positive by the rapid culture method. This indicates an appreciable superiority of this rapid culture method over the direct smear and concentrate techniques in this series.—*Cultivo rápido de bacilos ácido alcohol resistentes a partir de material patológico, L. F. Bojalil & A. Monnier M., Rev. mex. de tuberc., May–June, 1952, 13: 42.—(F. Pérez-Pina)*

Middlebrook Test.—Sera from 151 patients with tuberculosis and 128 sera from normal persons were studied using the Middlebrook test for hemagglutination and hemolysis of tuberculin-sensitized cells. In addition, a Coombs test modified to determine hemolysis and a procedure to detect blocking antibodies were studied, using these sera. Hemagglutinins were found in a titer of 1:8 or greater in only 56.1 per cent of the tuberculous sera and 21.2 per cent of sera from normal persons with positive tuberculins and 10.5 per cent of sera from tuberculin-negative persons. Titers of hemolysins were slightly greater but correlated well with the hemagglutination studies. Although blocking antibodies were not detected, a Coombs hemolysin was found in nearly all patients with active tuberculosis, even though the Middlebrook hemagglutination and hemolysis tests were negative. With the antigens available, it is apparent that none of these tests is sufficiently reliable to be of real use in detecting or following the course of tuberculosis.—*Hemagglutinins and hemolysis for erythrocytes sensitized with tuberculin in pulmonary tuberculosis, W. Hall & R. Manion, J. Clin. Investigation, December, 1951, 30: 1542.—(S. Hadley)*

Hemagglutination Test.—Hemagglutination tests using sheep erythrocytes and collodion particles sensitized with tuberculin were performed on a variety of sera. As in other studies, there was a greater number of high titers among the tuberculosis sera but the number among the nontuberculosis sera was large enough to render the test of little practical value. There was no correlation between the presence or absence of agglutinins in nontuberculosis sera and skin reactivity to tuberculin. Repeated intracutaneous tuberculin test of 5 tuberculin-negative persons failed to raise the hemagglutination titers. In addition, the hemolytic modification of the test failed to show greater specificity.—*Agglutination of sensitized sheep erythrocytes and collodion particles by tuberculosis and normal sera, C. Coliver & G. Pilner, J. Clin. Investigation, February, 1952, 31: 238.—(S. Hadley)*

"Two-dimensional" Complement-Fixation Tests.—Purified Old Tuberculin was prepared by repeated precipitation at the iso-electric point and used for complement-fixation tests against the sera of tuberculous guinea pigs, rabbits, and patients. The tests were made simultaneously using serial dilutions of both antigen and antibody in a "two-dimension method," described by Boyd and Purnell (1944) as the "checker board method." The sera from 2 tuberculous guinea pigs gave field dimensions between an antigen titer at a dilution of 1:51,200 and an antibody titer at the dilution of 1:40 with an antigen-antibody ratio at the optimal proportion of approxi-

mately 320. For the sera of 8 tuberculous rabbits, the values were 1:512,000; 1:80, and 50 to 1,600, respectively. For the sera of 15 tuberculin-positive and 6 tuberculin-negative patients with tuberculosis, the values were 1:64,000; 1:160; and 100 to 4,800, respectively. Two of 8 tuberculin-negative patients gave very small fields. The field patterns for the rabbits were of Boyd's R type, suggesting two sorts of antigen-antibody systems; those for humans were of Boyd's H type. In the latter the antigen-antibody ratio decreased as the tuberculosis progressed and increased as healing occurred. The results seem to indicate that any serologic testing for tuberculosis should employ a "two-dimensional method" to avoid false negative results, which may occur when only the antigen or only the antibody concentration is varied.—*Studies on the antigenic substances of Old Tuberculin reacting in the complement fixation test, T. Sindo, K. Wakakura, and I. Komine, Japanese J. Exper. Med., April, 1952, 22: 97.*—(*L. B. Hobson*)

Dissociation Curve of Lung Tissue.—The importance of the carbon dioxide combining capacity of the lung in modifying the alveolar partial pressure of carbon dioxide had been suggested by previous work. The curve describing this process was determined directly by exposing the exsanguinated lungs of 13 dogs to various pressures of carbon dioxide and then determining their carbon dioxide content. This curve was found to resemble that reported for other tissues and resulted in a value of approximately 0.80 cc. per kg. of lung tissue change in carbon dioxide content per mm. of mercury change in pressure. This is just sufficient to produce a measurable effect on the composition of the alveolar air, modifying it in such a manner as to lessen the changes which occur during breathing, breath holding, and other respiratory maneuvers.—*CO_2 dissociation curve of lung tissue, A. B. DuBois, W. O. Fenn, & A. G. Britt, J. Appl. Physiol., July, 1952, 5: 13.*—(*W. J. Osher*)

Oxygen Dissociation Curve.—The oxygen dissociation curve was reinvestigated because: (*1*) there are two appreciably different curves now in use; (*2*) these curves, having been obtained from only one person, do not exclude the possibility of wide interindividual variation; (*3*) the *in vivo* equilibration might be different from that done outside of the body; (*4*) the accuracy of gasometric methods has been challenged, having given lower values than spectrophotometric methods. Fifteen healthy resting male subjects were exposed to gas mixtures containing various concentrations of oxygen, and arterial blood samples were taken. Saturation of these samples were determined by a gasometric and a spectrophotometric method; gas tensions were obtained by a microtonometric technique. The curves thus obtained showed a close agreement with the *in vitro* curve of Bock, closer than that of Dill. No difference was noted in the curves of the 15 subjects. The values for oxygen saturation obtained by the spectrophotometric method were consistently higher and had less random variation than those obtained by the gasometric method but the discrepancy between the two methods was not as large as has been reported. Smokers were found to have a lower value for both arterial oxygen saturation and oxygen tension, the former possibly being due, in part, to inactive blood pigments.—*Relationship of oxygen tension to hemoglobin oxygen saturation in the arterial blood of normal men, C. J. Lambertsen, P. L. Bunce, D. L. Drabkin, & C. F. Schmidt, J. Appl. Physiol., June, 1952, 4: 873.*—(*W. J. Osher*)

Altered Pulmonary Function.—From a hospital population, 19 persons of various ages were selected as having no clinical or electrocardiographic evidence of lung or heart disease. Data obtained from these subjects were used to calculate the total lung volume, residual air, maximum breathing capacity, arterial oxygen and carbon dioxide concentration, dead space to tidal air ratio, estimated venous admixture, and the oxygen diffusing capacity of the lungs. Eight of the subjects had a sufficiently large ratio of residual air to total capacity to be classified as emphysematous and this group also had an abnormal relationship of dead space volume to tidal air, as did 6

of the remaining subjects. In 9 of the subjects there was some abnormality in either the estimated venous admixture, the alveolar oxygen diffusing capacity, or both. It was shown that a relatively high incidence of emphysema could occur at an early age and, in contrast to the obstructive type of emphysema, no abnormality of the maximum breathing capacity was present.—*Derangements of pulmonary function in individuals without clinical evidence of disease of heart or lungs, M. Galdston, W. B. Wolfe, & J. M. Steele, J. Appl. Physiol., July, 1952, 5: 17.*—(*W. J. Osher*)

Alveolar Carbon Dioxide and Oxygen.—The changes in alveolar carbon dioxide and oxygen during the respiratory cycle were considered from the standpoint of the effects of pulmonary blood flow and ventilation. The effect of the first factor was investigated by noting the effects of suspension of ventilation during a breath-holding period of twenty seconds and that of the latter by noting the diluting effect of a large inspiration of air. The data, plotted as an exponential curve, described the relationship between alveolar partial pressure of oxygen and carbon dioxide. The initial slope of this curve theoretically might be determined by the respiratory exchange ratio. The data showed a deviation from this slope which was due to the carbon-dioxide-combining capacity of the lung tissue. Similarly, a deviation was found from the expected result for the dilution curve due to the same effect. Calculations, described in a previous paper, were made of the partial pressure of the alveolar carbon dioxide and oxygen during the respiratory cycle. The values for these could be represented by a counterclockwise loop on the diagram of the alveolar partial pressures of oxygen versus carbon dioxide, the expiratory limb of this loop corresponding to a short period of breath holding and the inspiratory loop to the effect of dilution. The points on this loop could have been determined more accurately if the variations of pulmonary blood flow during the respiratory cycle were better known. The point for the mean alveolar partial pressure of oxygen and carbon dioxide was enclosed by this loop and could practically be attained most closely by taking a sample shortly after mid-expiration.—*Alveolar CO_2 and O_2 during breath holding, expiration, and inspiration, A. B. DuBois, J. Appl. Physiol., July, 1952, 5: 1.*—(*W. J. Osher*)

Hypoxia and Pulmonary Edema.—The role of hypoxia in the production of pulmonary edema was investigated by subjecting guinea pigs to oxygen-nitrogen mixtures in which the oxygen concentration was progressively decreased. The onset of the signs indicative of progressive anoxia was watched and, just before the expected time of circulatory failure, the animal was sacrificed. The lungs were analyzed in regard to their weight and the content of soluble protein nitrogen, insoluble protein nitrogen, and hemoglobin. A comparison of these values with those obtained from a control group indicated that there was no statistically valid difference. Hypoxia alone thus did not appear to produce any significant degree of pulmonary edema.—*Pulmonary edema in guinea pigs during severe hypoxia, A. Hemingway, J. Appl. Physiol., May, 1952, 4: 868.*—(*W. J. Osher*)

Vital Capacity at High Altitudes.—A reduction of the vital capacity was demonstrated in subjects when they were exposed in an altitude chamber to equivalent altitudes of 10,000 to 18,000 feet. The administration of oxygen under these conditions increased the vital capacity. A more marked reduction was found at higher altitudes even though oxygen was breathed. These reductions were not as large as those found by other authors but the discrepancy probably can be explained by the failure of the latter to correct the spirometer values for temperature and vapor pressure. Breathing low oxygen mixtures at ground level had a barely significant effect in reducing the vital capacity but did cause a definite diminution of the maximum force exerted by the expiratory muscles. The reduction of vital capacity at higher altitudes appeared to be best explained by distention of gases within the abdomen resulting in interference with movement of the diaphragm. Exposure to an

altitude of 14,000 feet for seven days resulted in a maximum decrease of approximately 4 per cent in the vital capacity on the third day, followed by a recovery to more than the original volume.—*Vital capacity at reduced barometric pressure, H. Rahn & D. Hammond, J. Appl. Physiol., March, 1952, 4: 715.—(W. J. Osher)*

Pharmacology of Bronchi.—The volumes of the bronchi, alveoli, and trachea were measured in dogs simultaneously by the methods Konzett-Rössler and Kahn. The simultaneous measurements prevent wrong conclusions due to the interference of circulatory factors which may change the concentration of carbon dioxide in the alveolar air. The bronchodilating effects of aludrine, buscopan, and effortil (ethyl-adrianol) were studied. Buscopan, which has a bronchodilating effect, prolongs aludrine's action. Effortil has a weak bronchodilating effect; if combined with aludrine, the effect is prolonged. In bronchospasm caused by histamine, the action of aludrine can be prolonged by effortil more significantly. Of all the drugs that were tested, aludrine and epinephrine are most effective against various bronchospastic substances.—*Gleichzeitige Anwendung zweier Methoden zur Untersuchung der Bronchialweite und zur Prüfung bronchoaktiver Suststanzen, W. Graubner, G. Peters, & H. Wick, Wien. klin. Wchnschr., August 29, 1952, 64: 635.—(G. C. Leiner)*

Respiratory Dead Space.—The idea, originally proposed by Haldane and his associates, that the respiratory dead space increased with the tidal volume and was a constant proportion of it was re-examined. A new method was used to calculate the dead space based on the fact that, if the alveolar air composition could be maintained constant for all tidal volumes, the Bohr formula for calculating the dead space could be simplified in such a manner that the expired air composition would be a linear function of the reciprocal of the tidal volume. By extrapolating this linear function in both directions to the point where the inspired and expired gas composition are equal and to the point where the reciprocal of the tidal volume is zero, the actual volume of the dead space and the composition of the alveolar air, respectively, could be obtained. In experimental practice, it was possible to maintain a constant arterial oxygen content in artificially ventilated dogs and in trained human subjects and from this it was assumed that the effective alveolar gas composition remained constant. The linear relationship between the expired air composition and the reciprocal of the tidal volume was then confirmed experimentally for oxygen and carbon dioxide and it was shown that the dead space was constant for the two gases and independent of the tidal volume. This method, furthermore, provided a means for measuring the alveolar air composition indirectly and was free of the errors associated with collecting samples. Utilizing this method it was shown that there was no significant alveolar-arterial oxygen gradient in dogs when their arterial oxygen saturation ranged from 57 to 85 per cent.—*New experimental methods for determination of effective alveolar gas composition and respiratory dead space, in the anesthetized dog and in man, J. R. Pappenheimer, A. P. Fishman, & L. M. Borrero, J. Appl. Physiol., May, 1952, 4: 855.—(W. J. Osher)*

Airway Resistance.—Pneumotachographic records were taken of normal subjects and subjects having impairment of respiratory muscles due to poliomyelitis. The resistance of the airways of these subjects could be made less by changing their attitude of breathing, that is, by opening the mouth during nasal breathing or by hyperextending the neck and assuming the yawn position. The tidal volume increased in both the normal and abnormal subjects with the mouth-open position and was even greater with the yawn position. This was also true when respiration was assisted with a body respirator adjusted to give various degrees of negative pressure. The best approach to linearity between the pressure gradient induced by the respirator and the tidal volume was found with the yawn position.—*Effect of attitude of breathing upon tidal volume,*

L. F. Peltier & M. B. Visscher, J. Appl. Physiol., June, 1952, 4: 901.—(W. J. Osher)

Abdominal Muscles in Respiration.—The electromyographic activity of the external oblique and the rectus abdominis muscles was studied during various forms of breathing in supine human subjects. During quiet breathing, some slight activity of these muscles was noted in 4 of the 10 subjects, occasionally in relation to the phases of respiration. Bursts of higher levels of activity were noted in all subjects during maximal voluntary inspiration and expiration. When the volume of expiration was increased by rebreathing from a spirometer, there was likewise considerable activity of the abdominal muscles but this was not seen associated with increased inspiration from this source. It appeared that the abdominal muscles were an important factor in limiting voluntary maximal inspiration, but not expiration, during hyperpnea. During expiration, the muscles were recruited near the end of the process, suggesting that they help to complete expiration. Ventilation of large volumes (40 to 60 liters per minute) was possible before these muscles were employed as accessory expiratory muscles.—*An electromyographic study of the role of the abdominal muscles in breathing, E. J. M. Campbell, J. Physiol., June 27, 1952, 117: 222.—(W. J. Osher)*

Pulmonary Vasomotor Responses.—Isolated cat lungs were perfused with the animal's own blood at a constant flow and changes in blood pressure were taken as an index of vasomotor activity. A pressor response, as has been described previously, took place on ventilation of the lungs with pure nitrogen and a fall in pulmonary blood pressure, usually accompanied by bronchodilatation, was noted with pure carbon monoxide or a mixture of 0.55 per cent carbon monoxide in air. These changes could be produced after dilution of the blood with dextran, suggesting that the partial pressure of these gases rather than the quantity was the important factor. The effect of carbon monoxide was not altered by atropine, dihydroergotamine, or decamethonium iodide. The response to nitrogen could be superimposed on that to carbon monoxide, suggesting that different mechanisms were involved. The possibility of the action of carbon monoxide by combining with heme compounds was suggested by the similarity of action of sodium cyanide and sodium azide, known inhibitors of heme-containing systems. —*Pulmonary vasomotor responses of isolated perfused cat lungs to anoxia, H. N. Duke & E. M. Killick, J. Physiol., July 28, 1952, 117: 303.—(W. J. Osher)*

Mineral Oil in Lung.—The positive identification of mineral oil in post-mortem examinations of lung tissue is of interest in the diagnosis of lipoid pneumonia. Staining of sections with Scarlet red (Sudan IV) is nonspecific and does not serve to distinguish mineral oil from all other fats occurring in the body. A simple method for examining lung tissue for mineral oil involves extraction with petroleum-ether, elimination of any interfering lipoid material by treatment with sulfuric acid, and the final identification of mineral oil by its physical and chemical properties. Specimens of normal and pathologic human lung, either freshly excised or preserved in formalin, were tested. The pathologic lung specimen was shown to contain an oil or lipoid material by staining with Sudan IV, and a diagnosis of lipoid pneumonia was established by post-mortem examination.—*Chemical examination of lung tissue for mineral oil, L. C. Donnelly & B. P. Hecht, Am. J. Clin. Path., May, 1952, 22: 449.—(J. S. Woolley)*

PUBLIC HEALTH AND EPIDEMIOLOGY

Statistical Trends in Tuberculosis.—The rapidly changing picture of the tuberculosis problem in the United States is emphasized when trends are reviewed statistically. (*1*) When tested at the age of sixteen years, 84 per cent of the children in Philadelphia had positive tuberculins in 1929, 43 per cent in 1939, and 16 per cent in 1949. (*2*) A total of 118,414 new cases of tuberculosis were reported in the United States during 1951. The reported case rates for white and non-white

males were, respectively, 86.6 and 194.8 per 100,000; and for white and non-white females, 49.9 and 136.1. There were 4 new cases reported for each death recorded. (*3*) In 1950, there were 33,577 tuberculosis deaths, or 2.3 per cent of the total deaths from all causes. (*4*) In 1952, men mostly in the older age group comprised 75 per cent of the sanatorium population. (*5*) At the Henry Phipps Institute in Philadelphia in the twenty-year period 1926 through 1945, patients with active disease diagnosed as minimal lived an average of 25.1 years after diagnosis; those in the moderately advanced stages, 9.9 years; those in the far advanced stage, only 1.1 years. With the improved case-finding program (13,000,000 chest roentgenograms were taken in 1951), increased number of beds, and greater use of chest surgery and chemotherapy, these encouraging advances will undoubtedly continue.—*The tuberculosis problem, R. Teague, Pub. Health Rep., August, 1952, 67: 759.*—(*S. Hadley*)

Yorkshire Tuberculosis Survey.—In the Calder Valley area of Yorkshire, England, 58.74 per cent of the children react to tuberculin when they reach the age of fourteen. Within the area there was no significant difference in the incidence of reactors between the sexes or among children attending urban, rural, or mixed schools or those residing in homes in urban, rural, or mixed areas. This incidence was 9 to 19 per cent higher than that in similar age groups in the cities of Hull and Leeds. The difference may have arisen because 40 per cent of the cows in the area are tuberculin-positive and only a small proportion of the milk is pasteurized. The number of reported cases of primary abdominal tuberculosis per 100,000 population was higher in Calder Valley than in Hull or Leeds. In the five- to seven-year-old groups, the Mantoux 1:100 intradermal test produced only 3 to 6 per cent more reaction than the tuberculin jelly test. Among the groups eight to fourteen years old, the increased incidence was 7 to 11.4 per cent. Roentgenographic evidence of active primary or pulmonary tuberculosis was noted in 0.16 per cent of children in the Calder Valley. A lower incidence of respiratory tuberculosis and primary calcification was noted in the Calder Valley than in the Leeds survey.—*Tuberculous survey in school children in the Calder Valley, S. Keidan, J. Lyons, B. Mann, & G. A. Wilthew, Brit. M. J., June 28, 1952, No. 4773: 1390.*—(*E. A. Riley*)

Reinfection Tuberculosis.—In order to evaluate the role of exogenous reinfection tuberculosis, morbidity was studied in the employees of a large French Sanatorium between 1942 and 1948. Known arrested tuberculosis was present in 151 employees, while 170 had a negative history for tuberculosis. Of the former, only those persons who had worked for a minimum of two years without interruption were included in the study. Also excluded were persons with pneumothorax effusions and very extensive pulmonary lesions even though they appeared stabilized. Among non-tuberculous employees, nonreactors to tuberculin and persons with recent tuberculin conversion were excluded; information on tuberculin sensitivity, however, was not available in all cases. In all tuberculous employees, the lesions were considered stable and inactive at the time of employment; however, verification by tomography and guinea pig inoculation was not always done and latent tuberculous activity may have been overlooked in some instances. Employees were divided in groups according to the degree of exposure to tuberculosis. The roentgenographic manifestations of relapse in the known cases occurred (*1*) at the site of the old lesions (7 cases) and were considered definitely of endogenous origin; (*2*) in previously uninvolved parenchyma with no evidence of activity at the site of the old lesion (5 cases) so that exogenous origin was believed to be extremely probable; (*3*) in more complex fashion so that the relationship between relapse and old sequelae was harder to evaluate (5 cases). In the latter, proximity of new and old lesions and lack of tomographic examinations cast doubt on the true arrest of the older lesion; in these cases, exogenous origin was possible but more con-

jectural. These 17 cases of relapse represent 11.1 per cent of the 151 employees with known tuberculosis (1.54 per 100 person-years). Under identical working conditions, 9 cases of tuberculosis occurred among the employees with no previous history of tuberculosis, representing an incidence of 4.9 per cent and 0.60 per 100 person-years. There was no mortality in this group. Comparing these data and taking into account the different risks of exposure, the following figures are arrived at: (*1*) Relapse appearing roentgenographically as endogenous according to the above description is not more frequent in employee groups with massive exposure than in the least exposed groups (0.74 per 100 person-years as compared with 0.88). (*2*) Clinically active tuberculosis appearing roentgenographically as probably exogenous was only observed in the most exposed group, i.e., the nurses; (*3*) Total morbidity of exogenous reinfection in all employees was 0.74 per 100 person-years in the most exposed groups. In nontuberculous employees the incidence was 6.74 per cent among the most exposed as compared with 2.38 per cent in the least exposed. While these figures are admittedly statistically not significant, they seem to point in the same direction. Finally, the incidence of tuberculosis considered exogenous roentgenographically was comparable in tuberculous and nontuberculous employees (0.9 and 0.6 per cent, respectively). There was a statistically significant difference in the incidence of all new tuberculous episodes in the tuberculous and nontuberculous employees (2.5:1); this was attributed to the role of endogenous relapse. These data confirm the radiologic distinction of exogenous and endogenous lesions. No conclusion can be drawn from this material as to greater sensitivity of patients with arrested tuberculosis to exogenous reinfection; this possibility has to be kept in mine.—*Le problème des reinfections exogènes chez les anciens tubercleux, étude comparé de la morbidité tubercleuse chez les employés de Sanatorium, anciens phtisiques ou non, G. Rocher & H. Seguin, Rev. de la tuberc., 1952, 16: 482.*—(*V. Leites*)

Tuberculosis Epidemic.—An epidemic of progressive pulmonary tuberculosis occurred in a rural high school in New York State. Among 133 pupils enrolled during a single school year, 13 cases of pulmonary tuberculosis were discovered within the succeeding two-year period. The ages of the pupils at the time of diagnosis ranged from 16 to 21 years. A 23-year-old teacher associated with them was also found to have the disease. Her age and clinical and laboratory findings suggest that rather than being the source case her case may well have been secondary. During the subsequent eight-year period, tuberculosis developed in 2 additional students in this group and one from the next lower class who entered the high school the following year. All cases were active progressive pulmonary tuberculosis and were hospitalized. There were 3 deaths. Two patients are still hospitalized. The remaining 12 patients are inactive following various forms of treatment. There was no significant familial exposure to tuberculosis except possibly in one case. The probable source case was a student whose disease was discovered one month after graduation and whose infection could be traced to an extrafamilial source outside the school. No case of tuberculosis was found in a community-wide roentgenographic survey in the area served by the school.—*Epidemic of tuberculosis in a high school: Report of eight year follow-up of students exposed, R. Horton, R. D. Champlin, E. F. H. Rogers, & R. F. Korns, J. A. M. A., May 24, 1952, 149: 331.*—(*H. Abeles*)

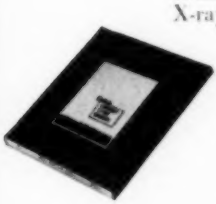
Tuberculin-Jelly Testing.—Simultaneous tests on 999 children, using Old Tuberculin jelly stored in a refrigerator and at room temperature for eleven to fifteen months gave only 4 discrepant results on the fifth day, which showed that the jelly could be kept at room temperature without losing potency. A series of tests, using OT jelly and PPD jelly simultaneously, confirmed earlier reports that OT jelly gives a higher proportion of positive results on the third day than does PPD jelly; the differences between the two being 3.6 per cent on the third day, 1.3 per cent on the

fifth day, and 0.8 per cent on the sixth day. The earlier unsatisfactory results with PPD jelly were due to the delayed positive reactions. Tests using OT jelly alone showed progressively more frequent positive results up to the sixth day. On the sixth day, one test previously positive became negative and the reactions tended to fade. On the seventh day, 13 reactions previously positive became negative. When OT jelly is used, the results should be read on the fifth day or early on the sixth day. When read on the third day, there were so-called false positive tests due, perhaps, to reactions to the adhesive as well as false negative reactions. In serial readings on 746 persons, only one positive reaction became negative before the sixth day. This probably was a false positive result on the third day. In another series of 510 persons, only one reaction, which was positive on the fourth and seventh days, became negative on the fifth and sixth days. This technical error stresses the need for controls. Thus, among 1,256 persons, only one gave a result possibly inconsistent with the true tuberculin state when read on the fifth day.—*Technique of jelly (tuberculin) testing, J. B. Lendrum, Brit. M. J., September 20, 1952, No. 4785: 649.—(E. A. Riley)*

BCG Vaccination.—The value of BCG vaccine in controlling tuberculosis was determined in groups of Indians in the United States and Alaska. School and preschool age children, all of whom were negative to tuberculin, were placed by an alternate division of cards into one of two groups. These groups were comparable in numbers, age, and sex. The members of one group received a single intracutaneous injection of freshly prepared BCG vaccine and were not revaccinated during the study. The second group received an intracutaneous injection of isotonic sodium chloride solution and served as controls. Neither group was isolated before or after vaccination, and no change was made in their mode of living. The study began between 1936 and 1938 and was terminated in 1951. Both groups were followed to the same degree annually for the first nine to eleven years of the study by means of tuberculin tests and roentgenologic examination of the chest. The vaccinated group consisted of 1,551 persons, of whom 1,534 were traced at the end of the study; they were observed for 21,245.5 person-years. The control group consisted of 1,457 persons, of whom 1,444 were traced; they were observed for 19,520.5 person-years. During the thirteen to fifteen years of observation, a total of 71 vaccinated and 128 controls died from all causes. Among the vaccinated persons, 12 died from tuberculosis, a rate of 0.56 per 1,000 person-years of observation. Among the controls, 65 died from tuberculosis, a rate of 3.32 per 1,000 person-years of observation. Among the vaccinated persons and controls, 59 and 63, respectively, died from nontuberculous causes. Of the 12 deaths from tuberculosis among the vaccinated group, 6 were due to pulmonary tuberculosis, 2 to bone and joint tuberculosis, 3 to tuberculous meningitis, and one to tuberculous meningitis and pulmonary tuberculosis. Among the nonvaccinated persons, 32 died after pulmonary tuberculosis, 2 from bone and joint tuberculosis, and a total of 31 from acute miliary or similar forms of tuberculosis, including tuberculous peritonitis. Hypersensitivity among the vaccinated group ranged from 93.3 per cent one year following vaccination to 90.2 per cent eleven years later. Among the controls, hypersensitivity increased from an incidence of 12.7 per cent one year after the initiation of the study to 41.7 per cent after eleven years. The observed data support the concept that there is a close, but not absolute, relationship between resistance to reinfection and hypersensitivity.—*Appraisal of protective value of BCG vaccination, J. D. Aronson & C. F. Aronson, J.A.M.A., May 24, 1952, 149: 334.—(H. Abeles)*

For superior radiographic results, follow this simple rule:

Use
Kodak Blue Brand
X-ray Film



Expose with
Kodak Contact X-ray
Screens (THREE TYPES)

Process in
Kodak Chemicals
(LIQUID OR POWDER)

Order from your x-ray dealer

His goal is exactitude . . .

Whether at home, absorbed in his hobby, or at his office, busy with patients, the radiologist is concerned with every detail. His demand is for accuracy, exactitude.

That's why, without a doubt, he requires the finest, most advanced equipment, the most responsive and reliable x-ray film, the most uniform and effective processing chemicals. And that's the reason he almost always specifies Kodak x-ray materials . . . each product made to work with the other, each prepared to produce superior results.

EASTMAN KODAK COMPANY
MEDICAL DIVISION, ROCHESTER 4, N. Y.

Kodak
TRADE-MARK

a broad spectrum dietary supplement

When anorexia interferes with the intake of needed foods in adequate amounts, the resultant effect on the nutritional status of the tuberculous patient is considerably more apt to involve deficiency in several nutrients than in one particular nutrient. As a consequence, unpredictable subclinical deficiency states may arise which predispose to a questionable prognosis. The rationale of prophylactic therapy against nutritional deficiencies, therefore, calls for a dietary supplement of broad nutrient spectrum capable of improving the intake of many nutrients.

The dietary supplement Ovaltine in milk enjoys long-established usage in clinical practice. As shown by the appended table, it supplies notable amounts of virtually all nutrients known to take part in metabolism. Its biologically complete protein provides an abundance of all the essential amino acids. It is delightfully palatable, easily digested, bland, and well tolerated.

Ovaltine is available in two varieties, plain and chocolate flavored, thus allowing choice according to preference. Serving for serving, both are virtually alike in their wealth of nutrients.

THE WANDER COMPANY, 360 N. MICHIGAN AVE., CHICAGO 1, ILL.

Ovaltine

Three Servings of Ovaltine in Milk Recommended for Daily Use Provide the Following Amounts of Nutrients

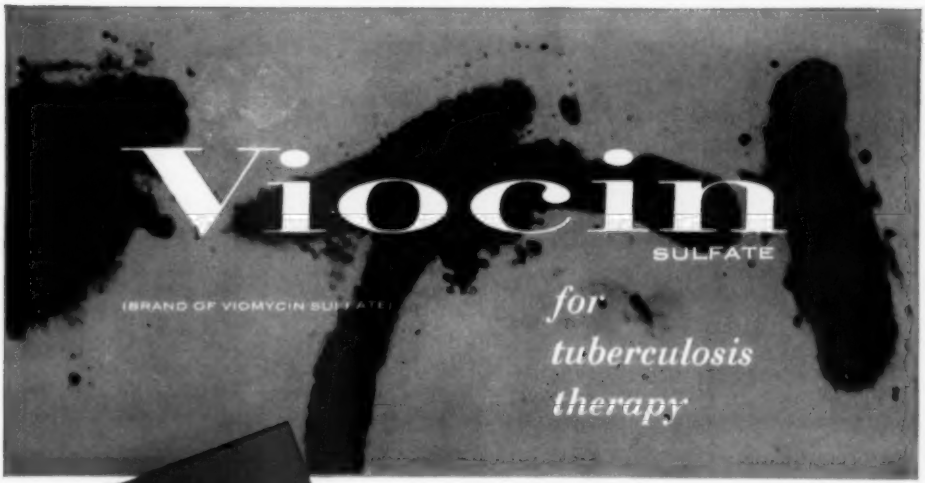
(Each serving made of ½ oz. of Ovaltine and 8 fl. oz. of whole milk)

MINERALS		VITAMINS	
*CALCIUM	1.12 Gm.	*ASCORBIC ACID	37 mg.
CHLORINE	900 mg.	BIOTIN	0.03 mg.
COBALT	0.005 mg.	CHOLINE	200 mg.
*COPPER	0.7 mg.	FOLIC ACID	0.05 mg.
FLUORINE	3.0 mg.	*NIACIN	6.7 mg.
*IODINE	0.15 mg.	PANTOTHENIC ACID	3.0 mg.
*IRON	12 mg.	PYRIDOXINE	0.6 mg.
MAGNESIUM	120 mg.	*RIBOFLAVIN	2.0 mg.
MANGANESE	0.4 mg.	*THIAMINE	1.2 mg.
*PHOSPHORUS	940 mg.	*VITAMIN A	3200 I.U.
POTASSIUM	1300 mg.	VITAMIN B_{12}	0.005 mg.
SODIUM	560 mg.	*VITAMIN D	420 I.U.
ZINC	2.6 mg.		

*PROTEIN (biologically complete)	32 Gm.
*CARBOHYDRATE	65 Gm.
*LIPIDS	30 Gm.

*Nutrients for which daily dietary allowances are recommended by the National Research Council.

a new achievement of *Pfizer* research



now released for clinical use

From the research laboratories of the world's largest producer of antibiotics comes Viocin — a new antibiotic with important activity against *Mycobacterium tuberculosis*.

A pioneer in the development of streptomycin, dihydrostreptomycin and isoniazid, Pfizer thus adds another powerful agent to the modern management of tuberculosis.

Viocin therapy will be particularly valuable when other medication cannot be used, and as an adjunctive measure in serious, progressive tuberculosis and many cases of extrapulmonary tuberculosis.

Viocin is supplied as the sulfate, for intramuscular injection.

For further details, see your Pfizer Professional Service Representative.

Pfizer ANTIBIOTIC DIVISION • CHAS. PFIZER & CO., INC.
BROOKLYN 6, N.Y.

Why Over 3,500 TB Case-Finding Projects Annually Use

POWERS X-RAY SERVICE

- **POWERS SERVICE IS COMPLETE!** Powers relieves project sponsors of *all* technical problems. Our experienced technicians work to the sponsor's schedule, with units able to take as many as 200 chest x-rays an hour. We deliver fully processed x-rays, with a viewer, to sponsor's roentgenologist.
- **LOWER IN COST!** The large volume of x-rays Powers handles nationally enables us to cut overhead costs to a minimum for each individual project.
- **EFFICIENT!** Our 20 years of experience in chest x-ray surveys from Maine to Texas have taught us to do the job quickly and effectively under many diverse conditions.
- **FLEXIBLE!** According to the sponsor's requirements, Powers offers either the full-size roll paper method or the 70 mm photofluorographic method—and operates either portable units or mobile units with generators.

Reg. T.M. POWERS X-RAY PRODUCTS

Our technical advisory service is available without obligation. Write before you plan a TB Case-Finding Project!

POWERS X-RAY PRODUCTS, INC.

Group Radiography

GLEN COVE, LONG ISLAND, N. Y.

THE *Syko* MATTRESS

—has been proved, by actual use in mental hospitals, to be practically indestructible without the use of a cutting edge. The coating actually toughens with time; also is highly resistant to ordinary causes of bed fires.

It is impervious to body liquids and non-irritating to the skin. As the Syko mattress can be cleaned with soap and water and disinfected without damage, the need for rubber or plastic covers is entirely eliminated. Cotton sheets will not crawl or wrinkle under the body.

Hospitals report—"they are what we have been needing" and "placing another and larger order for Syko mattresses".

We will replace any mattress that fails in the claims we make for it. Write, or send a trial order, direct to the factory office

The Rest-Rite Bedding Co.

Mattresses since 1898

207 North Main St. Mansfield, Ohio

POSITIONS AVAILABLE

RESIDENT PHYSICIAN—for active thoracic surgery service. Salary commensurate with experience, includes maintenance. Tuberculosis hospital. Apply State Tuberculosis Hospital Commission, New State Office Building, Frankfort, Ky.

RESIDENT PHYSICIAN, tuberculosis experience essential. Call or write Deborah Sanatorium, Brown's Mills, New Jersey.

MEDICAL DIRECTOR—50 bed tuberculosis hospital, Northeastern Ohio. Salary $10,000 plus maintenance. Experience in tuberculosis required. Apply Trumbull County Commissioners, Court House, Warren, Ohio.

SENIOR RESIDENT PHYSICIAN—Licensing in state required. American graduate with tuberculosis training, including care of surgical cases required. $6,000 per annum and maintenance. Apply Box 116, American Review of Tuberculosis, 280 Madison Avenue, N. Y. 16, N. Y.

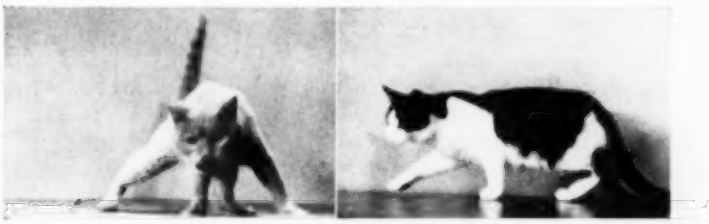
PHYSICIAN—with some experience in collapse therapy for private clinic in Detroit. Good salary and eventual partnership. Should be eligible for Michigan license. Apply Box 117, American Review of Tuberculosis, 280 Madison Ave., N. Y. 16, N. Y.

POSITION WANTED

CHEST SPECIALIST—CANADIAN—Also highly trained in internal medicine, with F.R.C.P., F.A.C.P., and F.C.C.P. Numerous publications. Desires responsible position. Research facilities and university affiliation mandatory. Apply Box 115, American Review of Tuberculosis, 280 Madison Avenue, N. Y. 16, N. Y.

New principle in Streptomycin Therapy

The hazard of ototoxicity is greatly reduced by combining equal parts of streptomycin sulfate and dihydrostreptomycin sulfate. The patient thus gets only *half* as much of each drug. The risk of vestibular damage (from streptomycin) and of hearing loss (from dihydrostreptomycin) is greatly reduced. Therapeutic effect is undiminished. This principle has been demonstrated in both animals and man. In patients treated for 120 days with 1 Gm. per day of the combined drugs, the incidence of neurotoxicity was practically zero.



Cat treated with streptomycin is ataxic. Cat treated with the same amount of streptomycin-dihydrostreptomycin has normal equilibrium.

DISTRYCIN
(di-STRI-sin)

Squibb Streptomycin Sulfate *and* Dihydrostreptomycin Sulfate in equal parts

For greater safety in combined antibiotic therapy
These new formulations embody this new principle:

	DICRYSTICIN	DICRYSTICIN FORTIS	DISTRYCILLIN A. S.
Procaine penicillin G, units	300,000	300,000	400,000
Potassium penicillin G, units	100,000	100,000	—
Streptomycin sulfate, Gm.	0.25	0.5	0.25
Dihydrostreptomycin sulfate, Gm.	0.25	0.5	0.25

(All supplied in 1 and 5 dose vials)

'Distrycin' and 'Dicrysticin' are registered trademarks; 'Distrycillin' is a trademark.

SQUIBB
a leader in the research and manufacture of penicillin and streptomycin

Thirty-ninth Session:

TRUDEAU SCHOOL OF TUBERCULOSIS

AT SARANAC LAKE, N. Y.

In June, 1953

Four Weeks, Course Beginning

JUNE 1ST

Schedule of Instruction includes history, etiology and epidemiology; pathology, bacteriology, physiology and biochemistry; clinics and bedside teaching, including surgical and special treatments; fungus infections; pneumoconioses; pulmonary tumors, and differential diagnosis of other lung disorders.

Clinical Facilities Available: Trudeau Sanatorium, Ray Brook State Hospital, Stony Wold Sanatorium, Will Rogers Memorial Hospital, Sanatorium Gabriels, V. A. Hospital at Sunmount, N. Y., and the General Hospital of Saranac Lake (chiefly surgical).

Research Facilities Available: Saranac Laboratory; Trudeau Laboratory; Physiology and Biochemistry Laboratories; Ray Brook State Hospital Laboratory, and the X-Ray Laboratories of Trudeau, Ray Brook and Stony Wold Sanatoriums.

Lectures, Demonstrations and Conferences conducted by the Staffs and other experienced local clinicians. By invitation, recognized authorities give lectures on specific subjects.

Students Participate Actively in the study and discussion of cases.

Fee for Four Weeks' Course is $100.00. The Trudeau School has been approved for training under Public Laws 610 and 550, and those desiring such an arrangement should clear their registration with the Veterans Administration before the session opens. The class is limited, therefore early application is advised. A few scholarships are available.

Note: Since 1939 the Trudeau School has been supported by grants from the Lillia Babbitt Hyde Foundation, of which Mr. Charles C. Harris is President.

For Schedule and other information write to

Secretary, TRUDEAU SCHOOL, Saranac Lake, N. Y.

NEW

NONTOXIC DETERGENT
FOR INHALATION...

ALEVAIRE
TRADEMARK

Liquefies

PULMONARY SECRETIONS
IN
Tuberculosis • Bronchial Asthma • Bronchiectasis
Bronchitis • Bronchopneumonia, etc.

Alevaire is an aqueous solution of a new nontoxic detergent, oxyethylated tertiary octylphenolformaldehyde polymer, 0.125 per cent, in combination with sodium bicarbonate 2 per cent and glycerin 5 per cent.

Alevaire is inhaled by means of any standard aerosol or nebulizer technic.

Life Saving in Neonatal Asphyxia

WRITE FOR INFORMATIVE LITERATURE.

Alevaire is also serviceable as a vehicle for penicillin, streptomycin,* decongestants (such as Neo-Synephrine®) or bronchodilators (such as Isuprel®).

Usual Dose: 500 cc. by aerosolization in 24 hours.
Available in bottles of 500 cc.

WINTHROP-STEARNS INC. NEW YORK 18, N. Y. • WINDSOR, ONT.

*Terramycin is incompatible.

Neo-Synephrine and Isuprel, trademarks reg. U.S. & Canada, brand of phenylephrine and isopropylarterenol, respectively.

739 M

New NTA Publication

Pennsylvania Pioneers

AGAINST

Tuberculosis

By Esther Gaskins Price

294 Pages *Cloth Bound* *$5.00 a Copy*

In this fully documented volume, there are brought together for the first time the stirring facts about the inception, development, and fruition of one of the most successful health crusades in history.

When this crusade started, tuberculosis was claiming human lives at the rate of more than 20,000 in a single decade in Philadelphia alone. Popular superstitions, medical conservatism, the hostility of landlords, industrialists and politicians, were some, but by no means all, of the difficult road blocks which confronted the pioneers.

Original Sources

Utilizing the proceedings of some of the most important medical meetings of the 19th century—the archives of the Pennsylvania Tuberculosis and Health Society and public health departments, newspaper files, and family records—PENNSYLVANIA PIONEERS AGAINST TUBERCULOSIS gives the historic details of the long drawn out battle waged by pioneers in the tuberculosis control movement.

This battle, spearheaded by Lawrence Flick and a small but forward-thinking group of earnest co-workers, finally led, among many other specific results, to the creation of the original tuberculosis society in the United States and the establishment of the first institution in medical history to concentrate on the prevention and treatment of a single illness.

Readers of the American Review of Tuberculosis will find in this book interesting and valuable background data, never before available, covering the critical period in which effective health control measures first began to assume their present shape.

Send your order today to:

National Tuberculosis Association, 1790 Broadway, New York 19, N.Y.

Additional postage outside U.S. and Canada, 50 cents a copy.

NATIONAL TUBERCULOSIS ASSOCIATION

ANNUAL MEETING **MAY 18–22, 1953**

LOS ANGELES, CALIFORNIA

The annual meeting of the National Tuberculosis Association will be held in Los Angles, California, May 18–22, 1953. The Hotel Statler and the Biltmore Hotel will be headquarters.

Some 1500 rooms have been promised by the hotels listed below for the accommodation of those attending the meeting. Arrange to share a twin bed room if possible.

Application for room reservations should be made directly to the hotel of your choice.

HOTEL	ADDRESS	SINGLE	TWIN	SUITES
Alexandria	210 W. 5th Street	$4.00– $8.00	$7.00–$10.50	$12.50–$25.00
Biltmore	515 S. Olive Street	$7.00–$11.50	$10.50–$14.50	$17.00–$33.00
Clark	426 S. Hill Street	$4.00– $6.00	$6.00–$ 8.00	$12.00–$18.00
Commodore	1203 W. 7th Street	$3.50–up	$6.00–up	$10.00–up
Hayward	206 W. 6th Street	$4.00– $6.00	$6.50– $8.00	$9.00–$15.00
Lankershim	230 W. 7th Street	$3.50– $5.00	$6.00– $7.50	$10.50
Mayfair	1256 W. 7th Street	$4.50–up	$7.50–up	$17.50
Mayflower	535 S. Grand Avenue	$5.25– $8.00	$5.75– $9.00	$14.00
Ritz Flower	813 S. Flower Street	$4.00– $5.00	$5.00– $6.00	—
Savoy	601 W. 6th Street	$4.00– $5.00	$5.50– $6.50	$8.00– $8.50
Statler, Wilshire and Figueroa		$6.50–$14.00	$10.00–$20.00	Not rated yet

IMPORTANT ANNOUNCEMENT

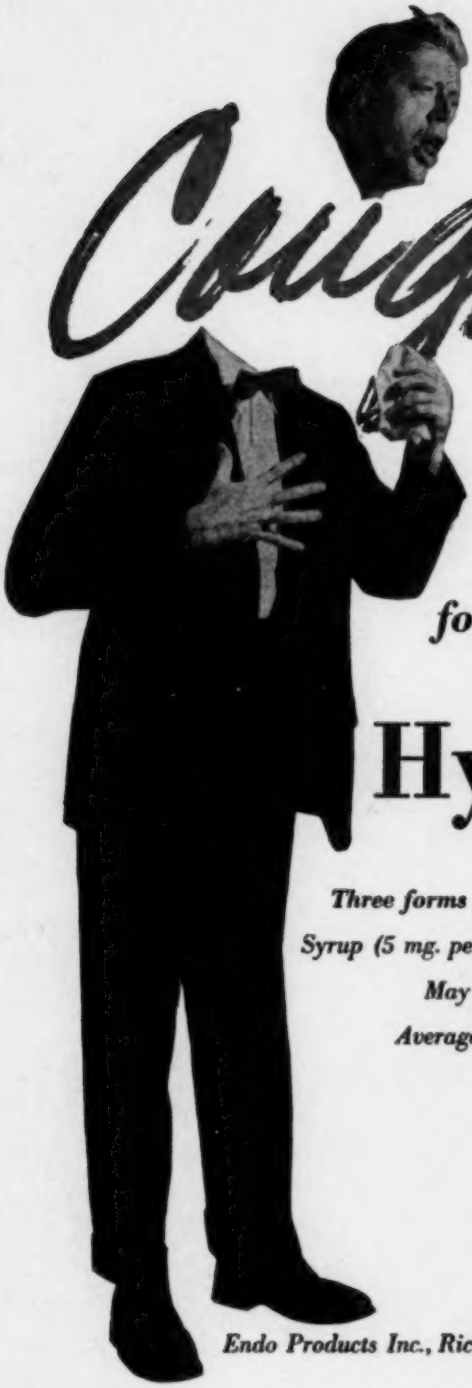
THE AMERICAN REVIEW OF TUBERCULOSIS has made available to its readers a section devoted to classified advertising pertaining to positions available and wanted. Since there have been so many requests for this type of service in the tuberculosis field, the Editorial Board of THE AMERICAN REVIEW OF TUBERCULOSIS decided to include a classified section at a nominal charge for each entry.

- Cost per advertisement of 25 words or less will be $3.50, additional words 15 cents each.
- Bold Face Semi-display will be $5.00 per insertion for 25 words or less, additional words 20 cents each.
- Post Office Box numbers will be an additional 75 cents per advertisement.
- Address all classified advertisements, with check or money order enclosed, to:

THE AMERICAN REVIEW OF TUBERCULOSIS

280 MADISON AVENUE **NEW YORK 16, N. Y.**

Murray Hill 5-8140



Coughing your head off!

for effective cough therapy

Hycodan® BITARTRATE

(Dihydrocodeinone Bitartrate)

Three forms available: Oral Tablets (5 mg. per tablet), Syrup (5 mg. per teaspoonful), Powder (for compounding).

May be habit forming; narcotic blank required.

Average adult dose 5 mg. Literature on request.

Endo®

Endo Products Inc., Richmond Hill 18, N.Y.